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Patterns of conjunctival and scleral regeneration after intraoperative application of cyclosporin A solution in rabbits with steroid-induced glaucoma

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ABSTRACT

Aim. In an *in vivo* experiment, to study the effect of local intraoperative application of 0.05% cyclosporin A solution on the conjunctival and scleral regeneration after surgery on the rabbit eyes with steroid-induced glaucoma.

Materials and methods. At the first stage of the experiment, a model of steroid-induced glaucoma was reproduced for 29 male Californian rabbits by injecting 0.5 ml of a 0.4% solution of dexamethasone subconjunctivally in both eyes once a week for 3 months (12 subconjunctival injections for each rabbit). At the second stage of the experiment, after the development of steroid-induced glaucoma, the rabbits were divided into the main group, consisting of the subgroup "a" ($n = 8$) and the subgroup "b" ($n = 8$), and the comparison group ($n = 8$). In all animals, a penetrating incision of the conjunctiva and a non-penetrating incision of the sclera of one of the eyes were performed.s. A hemostatic sponge impregnated with 0.05% cyclosporin A solution was applied to the intervention area in the main group, in the subgroup "a" – for 3 minutes, in the subgroup "b" – for 6 minutes. In the comparison group, the cytostatic was not used.

Results. The use of 0.05% cyclosporin A solution led to a decrease in the infiltration of fibroblasts and inflammatory cells in the area of surgical injury. On the 4th day after the surgery, cell density in the intervention area in the subgroup "a" with 3-minute application of cytostatic – antimetabolite solution was 2.7 times lower ($p = 0.043$) than in the comparison group, while exceeding the values in the subgroup "b" by 3.2 times ($p = 0.036$). The number of fibroblasts in the subgroups "a" and "b" was 3.6 ($p = 0.043$) and 12.8 times ($p = 0.031$) less than in the comparison group, and a shift in the cellular composition of the infiltrate towards the fibroblastic population occurred only on the 14th day after the surgery.

Conclusion. Intraoperative application of 0.05% cyclosporin A solution significantly slows down the course of regeneration, reducing infiltrative inflammation in the intervention area, which prevents excessive scarring.

Key words: antiglaucoma surgery, scarring of the outflow pathways of the intraocular fluid, antimetabolites, cyclosporine A.

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Закономерности регенерации конъюнктивы и склеры после интраоперационной аппликации раствора циклоспорина А у кроликов со стероидной моделью глаукомы

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РЕЗЮМЕ

Цель. В эксперименте *in vivo* изучить влияние местной интраоперационной аппликации 0,05%-го раствора (р-ра) циклоспорина А на регенерацию конъюнктивы и склеры после операции на глазах кроликов со стероидной глаукомой.

Материалы и методы. На I этапе эксперимента 29 самцам кроликов калифорнийской породы моделировали стероидную глаукому путем введения под конъюнктиву обоих глаз 0,5 мл 0,4%-го р-ра дексаметазона 1 раз в нед в течение 3 мес (12 инъекций). На II этапе эксперимента, после развития стероидной глаукомы, кроликов разделили на основную группу, состоящую из подгруппы «a» (n = 8) и подгруппы «b» (n = 8), и группу сравнения (n = 8). Всем животным выполняли сквозной разрез конъюнктивы и непроникающий надрез склеры одного из глаз. На область вмешательства в основной группе накладывали гемостатическую губку, пропитанную 0,05%-м р-ром циклоспорина А, в подгруппе «a» на 3 мин, в подгруппе «b» – на 6 мин. В группе сравнения цитостатик не использовали.

Результаты и обсуждение. Применение 0,05%-го р-ра циклоспорина А привело к уменьшению инфильтрации зоны хирургической травмы воспалительными клетками и фибробластами. На 4-е сут после операции клеточная плотность в области вмешательства в подгруппе «a» основной группы с трехминутной аппликацией р-ра цитостатика-антиметаболита была в 2,7 раза меньше (p = 0,043), чем в группе сравнения, превышая при этом показатели подгруппы «b» в 3,2 раза (p = 0,036). Численность фибробластов в подгруппах «a» и «b» была в 3,6 (p = 0,043) и 12,8 раза (p = 0,031) ниже, чем в группе сравнения. При этом сдвиг клеточного состава инфильтрата в сторону фибробластической популяции произошел только на 14-е сут после операции.

Заключение. Интраоперационная аппликация 0,05%-го р-ра циклоспорина А существенно замедляет течение воспалительно-репаративной регенерации, уменьшая инфильтративное воспаление в зоне вмешательства, что предотвращает излишнее рубцевание.

Ключевые слова: антиглаукомная операция, рубцевание путей оттока внутриглазной жидкости, антиметаболиты, циклоспорин А.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

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INTRODUCTION

According to modern concepts, glaucoma is a group of diseases characterized by intraocular pressure (IOP) above the tolerant level with development of optic neuropathy and a typical decline in visual functions [1]. Despite a wide choice of antihypertensive drugs and the use of various laser interventions, surgical treatment of this pathology remains the most effective method to compensate for increased IOP [1]. The success of filtration surgeries is determined by the duration of functioning of surgically created intraocular fluid outflow pathways. Scarring in the area of surgery leads to a significant decrease in the hypotensive effect [1, 2].

To provide a stable and prolonged hypotensive effect after antiglaucoma surgery, antimetabolites (5-fluorouracil, mitomycin C) are often used to prevent tissue proliferation in the intervention area with scarring of artificially created intraocular fluid outflow pathways [2–6]. However, widespread use of antimetabolites is limited by the risk of complications, such as secondary maculopathy, keratopathy, cataract progression, and hypotension, in the postoperative period [2].

Therefore, the search for a drug that has an antiproliferative effect with minimal toxic properties and prevents scarring of surgically created intraocular fluid outflow pathways after antiglaucoma surgery is one of the urgent problems of modern ophthalmic surgery.

The aim of the work was to study the effect of local intraoperative application of a 0.05% cyclosporin A solution on conjunctival and scleral regeneration after surgery on the rabbit eyes with induced glaucoma in an *in vivo* experiment.

MATERIALS AND METHODS

The study was carried out at the SSMU Laboratory of Biological Models (supervisor – Vladimir V. Ivanov, Cand. Sci. (Biology)). The protocol of the experiment was approved by the local Ethics Committee at SSMU (No. 4346 of 16.11.2015). The *in vivo* experiment was performed on 29 Californian rabbits (male), weighing 3.5–4.0 kg, which were preliminarily quarantined in the vivarium for 1 week on a standard diet. At the first stage of the experiment, the model of steroid-induced glaucoma was reproduced for all rabbits by injecting 0.5 ml of a 0.4% solution of dexamethasone (Belmedpreparaty, the Republic of Belarus) subconjunctivally in both eyes once a week for 3 months (a total of 12 subconjunctival injections for each rabbit) [7]. The IOP level in the rabbits was measured weekly using a Maklakov's tonometer with the plunger weighing 7.5 g. 2 weeks after the 12th injection of the steroid solution, 5 randomly selected animals were removed from the experiment, followed by enucleation of their eyes. The obtained material was fixed for light microscopy.

The second stage of the *in vivo* experiment was performed on 24 rabbits with steroid-induced glaucoma. 4 weeks after the last subconjunctival injection of 0.4% dexamethasone solution, in the upper part of the right eyeball of all the animals, a penetrating incision of the scleral conjunctiva and a non-penetrating incision of the surface layers of the sclera were performed under general anesthesia with sevoflurane (Baxter Healthcare SA, Puerto-Rico, USA) in the operating room according to aseptic and antiseptic rules. The paired eye of the animals remained intact.

Depending on the course of the surgery, the rabbits were divided into 2 groups: the main group (16 animals) and the comparison group (8 animals). During the surgery, after performing the incisions of the conjunctiva and the superficial layers of the sclera, a hemostatic sponge soaked in 0.05% cyclosporin A solution (Allergan, USA) was placed on the surgical area in all rabbits of the main group. Depending on the antimetabolite application time, the animals of the main group were divided into 2 subgroups: subgroup "a" (8 rabbits) – the application time of the drug was 3 minutes; subgroup "b" (8 rabbits) – the application time of the drug was 6 minutes.

A hemostatic sponge without cyclosporin A solution was placed on the surgical area for 3 minutes in the rabbits of the comparison group (8 animals). At the end of the application, the hemostatic sponge was removed in rabbits of all groups. The scleral conjunctiva incision was stitched with 2 interrupted sutures, and tobrex solution was instilled. In the postoperative period, all animals received one intramuscular injection of gentamicin sulfate solution (MICROGEN, Russia) at the rate of 5 mg / kg of the animal's weight, and tobrex solution was instilled into the conjunctival cavity of the operated eye 3 times a day. On days 4, 10, 14, and 21 after the surgery, 2 rabbits from each group were removed from the experiment, followed by enucleation of their eyes.

At all stages of the experiment, the animals were euthanized in compliance with the rules and norms of the European Society (86/609EEC), the Declaration of Helsinki, and orders of the Ministry of Health of the USSR (No. 742 of 13.11.1984 and No. 48 of 23.01.1985). The rabbits were removed from the experiment by carbon dioxide inhalation. The material

was fixed and then stained with hematoxylin and eosin and according to the Mallory technique for light microscopy at 100- and 200-fold magnification. Using Canon Power Shot G10 digital camera (Japan), 10 random fields of view were photographed for each slice. Digital photographs were subjected to morphometry using the ImageJ 1.46 program (http://rsbweb.nih.gov/ij/) and the Cell Counter plug-in, which were used to calculate the absolute and relative content of cells in the conjunctiva and sclera of the rabbits in the surgical area.

Statistical processing of the obtained data was carried out using the IBM SPSS Statistics 20 software. The Kolmogorov – Smirnov test was used to assess the normal distribution of the results. If the distribution of the indicators did not correspond to normal distribution, the Mann – Whitney U-test was used. The results were presented as $M \pm SD$, where M is the sample mean, and SD is the standard deviation. The differences were considered statistically significant at $p < 0.05$.

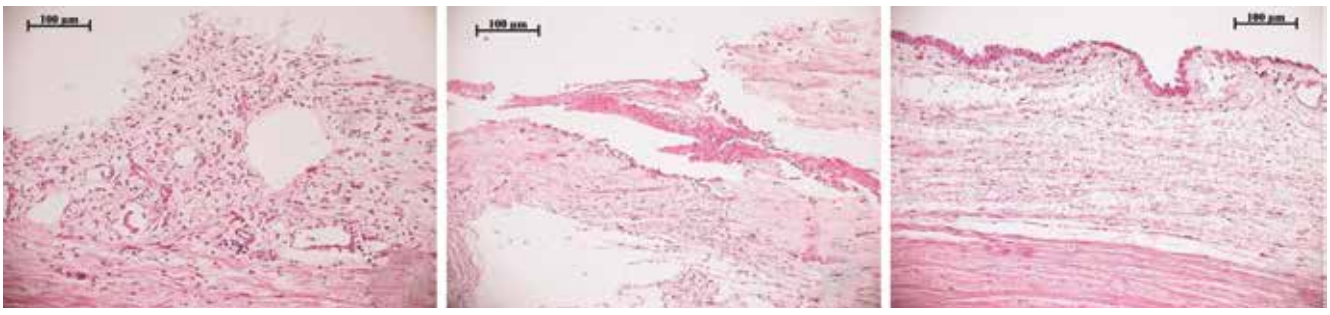
RESULTS

All rabbits (100%) had the initial IOP level within the normal range for this kind of animals, amounting to 13.7 ± 4 mm Hg [8]. After modeling steroidinduced glaucoma, all experimental animals showed an increase in the IOP level in both eyes by 3.5 times compared to the initial value − up to 47 ± 6 mm Hg (p = 0.041). Examination of the ocular fundus in all rabbits showed gradual expansion and deepening of the physiological excavation of the optic disc with the formation of glaucomatous excavation by the end of the 3rd month of the experiment, which was confirmed by the light microscopy findings. According to light microscopy, at the anterior chamber angle of the experimental animals, pathomorphological changes specific to glaucoma were revealed, such as partial lysis of the trabecular plates of the rabbit eye drainage system with the formation of dense conglomerates between the plates and obliteration of cracks between them. Areas of local ectasia of the Schlemm's canal were found, alternating with areas of its complete obliteration.

After instrumental and morphological confirmation of steroid-induced glaucoma development in the rabbits, the analysis of the second stage of the *in vivo* experiment started – the study of the effect of intraoperative application of 0.05% cyclosporin A solution on conjunctival and scleral regeneration of rabbits with induced glaucoma.

According to light microscopy, on the 4th day after the surgery, all rabbits from the subgroup "a" of the main group with intraoperative application of 0.05% cyclosporin A solution for 3 minutes had local conjunctival epithelium destruction in the surgical area. In the stroma, there were edema and fragmentation of collagen fiber bundles, between which multiple spaces were formed. In the sclera in the area of the surgical wound, the bundles of collagen fibers were located in mutually perpendicular directions, but the fibers were homogeneous and quite tightly adjoined each other, there were practically no gaps between them. Single vessels of the sclera were dilated and sanguineous (Fig. 1, *a*). Mononuclear leukocytes (MNL) prevailed in the cellular composition in the intervention area – 819 ± 54 cells (53.9%) in the field of view, the number of polymorphonuclear leukocytes (PML) and fibroblasts was 182 ± 24 (12.2%) and 516 ± 57 cells (33.9%) in the field of view, respectively.

In animals from the subgroup "b" of the main group, on the 4th day after thesurgery in the intervention area with the application of the antimetabolite solution for 6 minutes, pronounced damage to the conjunctival epithelium was detected. In the stroma, significant edema, fragmentation of collagen fibers, and multiple extravasal conglomerates of erythrocytes were detected. In the sclera, edema, hemorrhages, and fibrin clots were identified (Fig. 1, *b*).



a *b* *c*

Fig. 1. The state of the conjunctiva and sclera of the experimental animals in the area of intervention on the 4th day after the surgery: *a* – after a 3-minute application of 0.05% cyclosporin A solution, *b* – after a 6-minute application of 0.05% cyclosporin A solution, *c* – without application of the antimetabolite solution. Staining with hematoxylin and eosin, × 100

In the cellular composition, MNL prevailed – 283 ± 51 cells (59.1%). The number of PML was 53 ± 4 cells (33.9%) in the field of view. The number of fibroblasts was 143 ± 22 cells (29.8%), which is 3.6 times less than the level in the rabbits from the subgroup "a" of the main group ($p = 0.038$).

In the rabbits of the comparison group, on the 4th day after the surgery, in the intervention area without application of the antimetabolite solution, thinning and flattening of the conjunctival epithelium were found. In the sclera, edema and stratification of collagen fibrils were detected (Fig. 1, *c*). In the cellular composition, MNL and fibroblasts dominated with 1,961 ± 236 cells (47.0%) and 1,836 ± 218 cells (44.1%) in the field of view, respectively. The number of fibroblasts exceeded that in the rabbits of the subgroups "a" and "b" by 3.6 times ($p = 0.043$) and 12.8 times ($p = 0.031$), respectively. The PML density was 375 ± 15 cells (8.9%) in the field of view.

On the 10th day, in the animals from the subgroup "a" of the main group, in the intervention area with the application of 0.05% cyclosporin A solution for 3 minutes, local conjunctival defects were completely epithelialized due to stratified squamous epithelium. The stroma of the conjunctiva of the eye was loose and thickened due to diffuse edema; closer to the sclera, longitudinally located cavities were found in it, lined with a single layer of squamous epithelium. In the sclera in the area of intervention, the bundles of collagen fibers were stratified. However, these bundles looked structured. Fibroblasts predominated in the cellular composition – 948 ± 49 cells (68.4%) in the field of view, while their number increased by 1.8 times compared to the level on the 4th day after the surgery ($p = 0.038$). The number of PML was 21 ± 4 cells (1.5%) in the field of view, which is 8.9 times ($p = 0.046$) less than the level on the 4th day after the surgery. The density of MNL decreased by 2.0 times ($p = 0.037$) compared to the value on the 4th day after the surgery, amounting to 417 ± 36 cells (30.1%) in the field of view.

In the rabbits from the subgroup "b" of the main group, on the 10th day after the surgery with the application of 0.05% cyclosporin A solution for 6 minutes, the areas of the conjunctival epithelium destruction decreased, and the stroma of the membrane was extremely thinned. The conjunctival vessels of the operated eye were moderately dilated. In the sclera, the collagen fiber bundles were oriented parallel to each other, but had different thickness. Fibroblasts predominated in the cellular composition — 779 ± 35 cells (68.2%) in the field of view, which was 5.4 times ($p = 0.045$) higher than the level on the 4th day after the surgery. The cell density of the fibroblastic population was 1.2 times lower than in the rabbits from the subgroup "a" of the main group on the 10th day after the intervention ($p = 0.035$). The number of PML decreased by 2.2 times ($p = 0.042$) compared to the value in the animals of the subgroup "b" on the 4th day after the surgery, amounting to 24 ± 3 cells (2.1%) in the field of view. The number of MNL increased to 340 ± 17 cells (29.7%) in the field of view, which was 1.2 times higher ($p = 0.039$) than the level in the rabbits from the subgroup "b" on the 4th day after the operation.

In the rabbits of the comparison group, on the 10th day after the operation without application of 0.05% cyclosporin A solution, the conjunctival structure in the area of surgical injury appeared normal, collagen bundles in the sclera were thick and tightly adjoined each other. Fibroblasts predominated in the cellular composition – 3,174 ± 149 cells (80.0%) in the field of view, which was 1.7 times ($p = 0.034$) higher than their level on the 4th day after the intervention, as well as 3.3 times ($p = 0.031$) and 4.1 times ($p = 0.027$) higher than the values in the rabbits of the subgroups "a" and "b" on the 10th day after the surgery, respectively. The PML density decreased by 2.4 times ($p = 0.031$) compared to the level on the 4th day after the surgery, amounting to 159 ± 8 cells (4%) in the field of view. The number of MNL was 634 ± 31 cells (16%) in the field of view, decreasing by 3.1 times ($p = 0.043$) compared to their level on the 4th day.

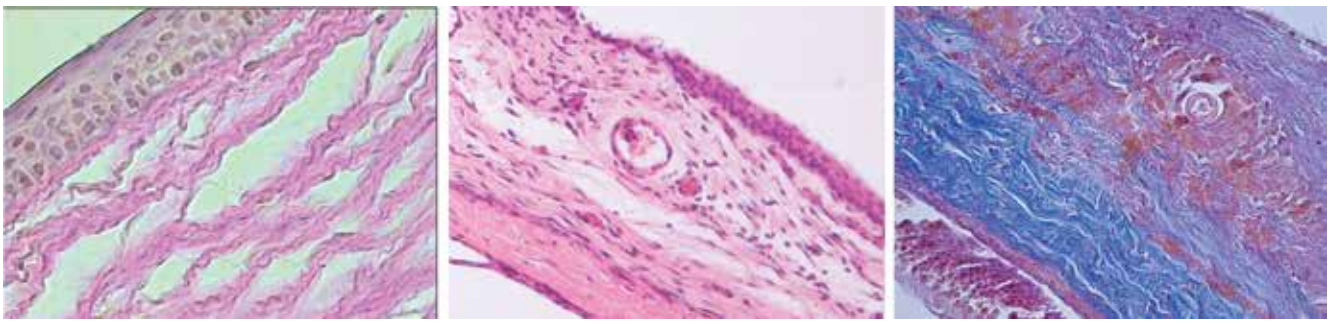
On the 14th day after the surgery, the animals of the subgroup "a" of the main group with the application of the antimetabolite solution for 3 minutes had a normal structure of the conjunctival epithelium in the intervention zone. However, in some regions, areas of surface layer thinning and thickening of the stroma due to residual edema were found. Closer to the sclera, in the conjunctival stroma, longitudinally located channels lined with a single layer of squamous epithelium were revealed. In the sclera, in the area of the surgical injury, stratification of collagen fiber bundles was observed. In the cellular composition, fibroblasts dominated – 1,311 ± 124 cells (69.5%) in the field of view, and their number increased by 1.4 times ($p = 0.035$) compared to the level in the rabbits of the subgroup "a" on the 10th day after the surgery. The density of PML was 84 ± 5 cells (4.4%) in the field of view, and their number increased by 4.1 times ($p = 0.039$) compared to the value in the animals of the subgroup "a" on the 10th day after the surgery.

The MNL level decreased to 492 ± 20 cells (26.1%) in the field of view, while there were no statistically significant differences from the level in the subgroup "a" of the main group on the 10th day after the surgery ($p = 0.062$).

In the rabbits of the subgroup "b", on the 14th day after the intervention with the application of the antimetabolite solution for 6 minutes, the conjunctiva in the area of the surgical injury was covered with stratified squamous epithelium, and slight subepithelial edema was found. The conjunctival stroma was loose. The collagen fiber bundles of the sclera in the area of intervention were loose. Fibroblasts predominated in the cellular composition with 867 ± 44 cells (65%) in the field of view, and there was no statistically significant difference ($p = 0.053$) from their density in the animals of the subgroup «b» on the 10th day after the surgical manipulation. The MNL level was 413 ± 32 cells (31%) in the field of view; there was no statistically significant difference ($p = 0.057$) compared to the value in the rabbits of the subgroup "b" on the 10th day after the surgery. The number of PML increased to 53 ± 2 cells (4%) in the field of view, exceeding by 2.2 times ($p = 0.029$) the value in the rabbits of the subgroup "b" on the 10th day.

In the rabbits of the comparison group, on the 14th day after the surgery without application of the antimetabolite solution, the conjunctiva of the eye in the intervention zone had a normal structure and tightly adjoined the superficial layers of the sclera. Scleral fibers were located tightly to one other. Among the cells in the area of the surgical injury, fibroblasts predominated with 1,972 ± 112 cells (84%) in the field of view, which exceeded the value in the subgroups "a" and "b" on the 14th day by 1.5 times ($p = 0.047$) and 2.3 times ($p = 0.038$), respectively. The density of PML decreased to 117 ± 5 cells (5%) in the field of view, which was 1.4 times (p = 0.047) lower than that on the 10th day. The MNL level was 258 ± 14 cells (11%) in the field of view, decreasing by 2.5 times ($p = 0.038$) compared to the indicator on the 10th day.

On the 21st day, in the animals of the subgroup "a" after application of 0.05% cyclosporin A solution for 3 minutes, the conjunctiva of the eye in the area of the surgical injury had a normal structure and was separated from the underlying sclera by a narrow slit-like space. The collagen fibers of the sclera were structured (Fig. 2, *a*). Fibroblasts predominated in the cellular composition with 839 ± 44 cells (69.7%) in the field of view, which was 1.6 times ($p = 0.036$) less than on the level on the 14th day after the surgery. The number of PML and MNL was 20 ± 2 cells (1.7%) and 344 ± 11 cells (28.6%) in the field of view, respectively. Moreover, the PML level decreased by 4.3 times ($p = 0.042$), and the MNL level – by 1.4 times ($p = 0.031$), compared to those on the 14th day.



a *b* *c*

Fig. 2. The state of the conjunctiva and sclera of the experimental animals in the area of intervention on the 21st day after the surgery: *a* – after a 3-minute application of 0.05% cyclosporin A solution, *b* – after a 6-minute application of 0.05% cyclosporin A solution, *c* – without application of the antimetabolite solution. Staining: *a, b* – with hematoxylin and eosin, × 100; *c* – according to the Mallory method, × 100

In the animals of the subgroup "b" of the main group on the 21st day after the surgery with the application of 0.05% cyclosporin A solution for 6 minutes, the conjunctiva of the eye in the area of the surgical injury had a normal structure and was separated from the underlying sclera by a narrow-slit space. In the conjunctival stroma, structured channels lined with a single layer of squamous epithelium were revealed. In the sclera, in the area of the surgical intervention, the collagen fiber bundles were loose, but they were oriented parallel to one another (Fig. 2, *b*). Fibroblasts predominated in the cellular composition with 799 ± 13 cells (69.3%) in the field of view, with no statistically significant differences ($p = 0.058$) compared to this indicator on the 14th day. The number of MNL decreased by 1.3 times compared to the 14th day, amounting to 320 ± 14 cells

(27.7%) in the field of view ($p = 0.044$). The number of PML decreased by 1.5 times ($p = 0.042$) to 35 ± 3 cells (3%) in the field of view.

In the rabbits of the comparison group, on the 21st day after the surgery without local application of 0.05% cyclosporin A solution, the conjunctiva of the eye in the area of the surgical injury had a normal structure and tightly adjoined the underlying sclera (Fig. 2, *c*). Fibroblasts predominated in the cellular composition – 1,209 ± 132 cells (83%) in the field of view. The cell density of this population decreased by 1.6 times compared to the value on the 14th day ($p = 0.045$), however, it exceeded this indicator by 1.4 times ($p = 0.048$) in the subgroup "a" and by 1.5 times ($p = 0.046$) in the subgroup "b" of the main group. The number of PML was 116 ± 5 cells (8%) in the field of view, with no statistically significant difference from the value on 21th day ($p = 0.059$). The number of MNL decreased by 2.0 times ($p = 0.037$) compared to the level on the 14th day, amounting to 131 ± 7 cells (9%) in the field of view.

DISCUSSION

According to the results of the experiment, the course of the inflammatory-reparative response in the conjunctiva and sclera in the rabbits of the comparison group with steroid-induced glaucoma after the eye surgery was characterized by a consistent change of cell phases in the surgical area with scarring of the conjunctiva and sclera on the 21st day. Local application of 0.05% cyclosporin A solution during the intervention had a significant effect on the postoperative period depending on the application time.

For example, a decrease in the migration of cells responsible for the inflammation intensity to the area of the surgical injury was revealed. On the 4th day after the surgery, the cell density in the intervention area in the subgroup "a" of the main group was 2.7 times lower ($p = 0.043$) than in the comparison group, while exceeding the value in the subgroup "b" by 3.2 times ($p = 0.036$) (Table). Similar dynamics was observed up to the 14th day after the surgery (Table).

Table

Dynamics of the cell density of the infiltrate in 1 mm² section in rabbits of the experimental groups with steroid-induced glaucoma, *M* ± *SD*

Observation period, days	Experimental groups		
	Main group		Comparison group
	Subgroup "a"	Subgroup "b"	
4	1,518 ± 51*/**	479 ± 22*	4,172 ± 181
10	1,386 ± 43*	1,143 ± 47*	3,967 ± 172
14	1,887 ± 84	1,333 ± 78*	2,347 ± 124
21	1,203 ± 54	1,154 ± 56	1,456 ± 97

* $p < 0.05$ when comparing to the values in the rabbits operated without local application of 0.05% cyclosporin A solution (comparison group); ** $p < 0.05$ when comparing the values in the subgroup "a" (application of the antimetabolite for 3 minutes) with the values in the subgroup "b" (application of the antimetabolite for 6 minutes) of the main group.

Intraoperative application of the antimetabolite had a pronounced inhibitory effect on the migration of fibroblasts to the area of the surgical injury. On the 4th day after the surgery, the density of fibroblasts in the intervention area in the subgroup "a" was 3.6 times lower ($p = 0.043$) than in the comparison group, while in the subgroup "b", it was 12.8 times lower ($p = 0.031$) than in the comparison group. On the 10th day after the surgery, the number of fibroblasts in the area of the surgical injury in the comparison group increased by 1.7 times ($p < 0.05$). In the subgroups "a" and "b" of the main group, a shift in the cellular composition in the intervention area towards the fibroblastic population occurred only on the 14th day after the surgery.

As a result, on the 21st day after the surgery, in the rabbits of the subgroup "a" of the main group, in the area of the surgical injury, only partial adhesion between conjunctiva and sclera was formed, in contrast to the animals of the comparison group. In the rabbits of the subgroup "b" of the main group, even on the 21st day after the surgery, a slit-like space between the conjunctiva of the eye and the underlying sclera remained in the intervention zone. Probably, the suppression of T-lymphocyte activation by cyclosporin A following the formation of bonds with cyclophilins in the cell cytoplasm and blocking of the catalytic and regulatory subunits of calcineurin leads to a decrease in the synthesis of proinflammatory cytokines. There-

fore, this slows down the course of the inflammatory-reparative response in the intervention zone [9].

CONCLUSION

Local application of 0.05% cyclosporin A solution during surgery on the conjunctiva and sclera of the eyes of rabbits with steroid-induced glaucoma significantly slows down the course of the inflammatory-reparative response in the intervention area, reducing the cell density by 2.9–3.5 times and preventing tissue scarring. The severity of the antiproliferative effect of the drug depends on the application time: when applied for 3 minutes, the density of fibroblast distribution in the surgical area is 3.6 times lower than in the rabbits of the comparison group, and when applied for 6 minutes, it is 12.8 times lower than in the comparison group. The obtained results are of interest for development of methods to prevent scarring after antiglaucoma surgeries.

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Authors contribution

Zhigalskaya T.A. – conception and design of the study, analysis and interpretation of data. Dzyuman A.N., Khlusov I.A. – analysis and interpretation of data. Krylova A.A. – analysis and interpretation of data, drafting of the article. Krivosheina O.I. – drafting of the article, substantiation of the manuscript, critical revision for important intellectual content, final approval of the manuscript for publication.

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