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MORPHOLOGICAL CHANGES IN HARDERIAN GLANDS AFTER THE IMPLANTATION OF POLYCAPROLACTONE FILMS INTO THE CORNEA

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МОРФОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ ЖЕЛЕЗ ГАРДЕРА ПОСЛЕ ИМПЛАНТАЦИИ ПЛЕНОК ПОЛИКАПРОЛАКТОННЫХ В РОГОВИЦУ

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Аннотация. *Имплантация пленок поликапролактона в строму роговицы глаза сопровождается развитием незначительных реактивных изменений желез Гардера в виде умеренного полнокровия сосудов, отека стромы железы, которые обусловливают неспецифические реактивные изменения в железе. Реактивные изменения железы Гардера (умеренное полнокровие сосудов, отек стромы железы) свидетельствуют об экзогенном механизме - хирургическом воздействии на морфологическую трансформацию железы.*

Introduction. Polycaprolactone (PCL) is a biodegradable polyester and is widely used in medicine: for tissue engineering, as substrates, microspheres and scaffolds for drug delivery systems, for the production of threads and pins [1]. Of particular interest is the use of PCL as a corneal implant for the bullous keratopathy treatment. The bullous keratopathy is the severe cornea disease and occupies one of the leading positions among the causes of corneal weak vision. Primary and secondary processes of the degenerative and infectious diseases of the cornea play a significant role in the development of bullous keratopathy, which leads to the death of endotheliocytes and dysfunction of the corneal endothelial layer. This contributes to the development of corneal edema, reduced vision, the occurrence of recurrent erosions and pronounced pain symptom.

Implantation of the PCL films for bullous keratopathy is possible in two ways: into the corneal stroma and into the anterior chamber of the eye. At present, the authors of the article have established that the implantation of the PCL films into the cornea or into the eye anterior chamber is accompanied by mild leukocyte infiltration and vasculogenesis in the cornea [2].

In addition, despite the abundance of literary sources on the use of PCL in medicine [3, 4], there is no information regarding the effect of this polymer on morphological changes in Harderian glands (HG) during implantation of the PCL films.

The purpose of this research is to determine the morphological changes in the HG after the implantation of PCL films into the corneal stroma of the eye.

Materials and methods. The feedstock for films was obtained by dissolving PCL with the molecular weight, Mw = 80,000 g/mol (Sigma-Aldrich, England) in the chloroform (CHCl3). 1% solution was poured into Petri dishes (12 g) which were placed in a fume hood until complete evaporation of $CHCl_3$ and the formation of PCL films.

9 pubescent male Sylvilagus bachmani rabbits (SSMU, Tomsk, Russia) weighing 2.5-3.0 kg were used. All animals were healthy and free of ocular diseases. This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Siberian Medical State University Life Science Ethical Review Committee (protocol № 7892 from May 13th, 2019) and conformed to the ARVO guidelines for the Use of Animals in Ophthalmic and Vision Research. Before the experiment, all the animals stayed in vivarium isolation with a regular feeding schedule for one week.

The animals were divided into 2 groups:

The 1st group (n = 3) – the intact (control) group.

The 2nd group (n = 6) – animals with an implanted PCL film with a diameter of 8.0 mm in layers of the cornea's own substance closer to the Descemet's membrane. Tobrex solution was instilled 3 times per day, 0.1% Diclofenac Sodium Ophthalmic Solution was instilled 3 times per day and 5% Corneregel was placed in the lower eyelid 2 times per day after the operation during 2 weeks.

The overall duration of the experiment comprised 30 days. Such methods as visual check, an optical coherent tomography (OCT) of cornea and photographic registration of the eye were also used in the course of the experiment. Sampling (HG, cornea) was performed on day 30 after the start of the experiment for morphology studying. Biological materials (HG, cornea) were fixed in 10% solution of neutral formalin and were embedded in paraffin according to the generally accepted method. Cross-sections of the HG were stained with hematoxylin and eosin. The counting and photographing of HG cross-sections were carried out at a magnification of 400 times using a Mikmed-6 microscope (LOMO, Russia) and an MPKS digital video camera (LOMO, Russia). The specific volumes (%) of the epithelium and stroma of the HG were calculated using Avtandilov's ocular insert. The epithelial/stromal ratio (ESR) was also calculated. The optical coherent tomography of cornea was carried out on Cirrus HD-OCT 5000 (Germany).

Statistical package IBM SPSS Statistics 20 was used for the statistical processing of the obtained results. Medians (Me) and interquartile range (Q1-Q3) were determined for biological data. Means (m) and standard deviations were determined for physical data. The nonparametric Mann-Whitney test was used to assess the differences. Values of $p<0.05$ were considered statistically significant.

Results. The effect of PCL films implantation on corneal changes was presented in the work [2]. Structural changes in the HG were expressed after the implantation of PCL films into the corneal stroma of the eye. The HG in the 2nd group was characterized by a high density of the secretory acini, between which there was loose connective tissue. The acini were expanded, lined with glandular cells of various shapes (from columnar to squamous). The expanded secretory acini of the HG contained lipid secretions in the lumen. The cytoplasm of the secretory acini had a fine-mesh structure. Cell nuclei were located in the basal regions. Full-blooded vessels and moderately pronounced edema were observed in the stroma of the HG.

The statistics analysis showed that the specific volumes of the epithelium and stroma in the 2nd group significantly differed from the 1st group. Specific volume of the HG epithelium in the 1st group was 44 (42-47)%, in the 2nd group was 42.5 (38.5-45)%. Specific volume of the stroma of the HG epithelium in the 1st

group was 4 (2-7)%, in the 2nd group was 5.5 (4-8.5)%. Analysis of ESR revealed a decrease of ESR in the 2nd group where it was 7.8 ($p < 0,05$). The ESR of the 1st group was 11.

According to the results, the implantation of the PCL films into the corneal stroma of the eye is accompanied by the development of minor reactive changes in the HG, such as moderate vascular congestion, edema of the gland stroma. This is probably due to increased secretion of the HG as indicated by the appearance of flattened forms of glandular cells. Thus, the implantation of PCL films causes nonspecific reactive changes in lacrimal HG to varying degrees of severity. The reactive changes in the HG (moderate vascular congestion, edema of the gland stroma) indicate an exogenous mechanism – a surgical effect on the morphological transformation of the gland.

Conclusion. The implantation of the PCL films into the corneal stroma of the eye is accompanied by the development of minor reactive changes in the HG, such as moderate vascular congestion, edema of the gland stroma, which cause with nonspecific reactive changes in the HG. The reactive changes in the HG (moderate vascular congestion, edema of the gland stroma) indicate an exogenous mechanism – a surgical effect on the morphological transformation of the gland.

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