

THE LEVEL OF LOCAL CYTOKINE PRODUCTION AT DIFFERENT TIMES AFTER EXPERIMENTAL CILIARY BODY CRYODESTRUCTION

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✧ We assessed the level of pro- (IL1 β , FNO α , IL2) and anti-inflammatory (IL10) cytokines in the anterior chamber fluid of experimental animals on the 3rd, 7th and 30th day, after an experimental ciliary body destruction with porous titanium nickelid cryoapplicator. Consistent changes of the cytokine content at the local level are reflecting the reactivity of the experimental animals as an answer both to surgery in ciliary body area, and to its experimental cyclodestruction using the cryoapplicator from titanium nickelid. The predominance of pro-inflammatory cytokines' potential was found during the early period (3–7 day), and the balance normalization of pro- and anti-inflammatory cytokines was found at late observation period (30 days) due to the increase of the anti-inflammatory IL-10 production; this effect was maintained at the end of the long-term study period (1.5 years).

✧ **Key words:** ciliary body; cryosurgery; titanium nickelid; experimental animals.

УРОВЕНЬ ЛОКАЛЬНОЙ ПРОДУКЦИИ ЦИТОКИНОВ В РАЗЛИЧНЫЕ СРОКИ ПОСЛЕ ЭКСПЕРИМЕНТАЛЬНОЙ КРИОДЕСТРУКЦИИ ЦИЛИАРНОГО ТЕЛА

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✧ Проведена оценка уровня содержания про- (ИЛ1 β , ФНО α , ИЛ2) и противовоспалительных (ИЛ10) цитокинов во влаге передней камеры глаза лабораторных животных на 3, 7 и 30 сутки после экспериментальной деструкции цилиарного тела криоаппликатором из пористого никелида титана. Установлено, что достоверные изменения содержания цитокинов на локальном уровне являются отражением реактивности организма лабораторных животных как на оперативное вмешательство в области цилиарного тела, так и на его экспериментальную циклодеструкцию криоаппликатором из никелида титана. Выявлено преобладание провоспалительного потенциала цитокинов в ранние (3–7 сутки) сроки, и нормализация баланса про- и противовоспалительных цитокинов в поздние (30 суток) сроки наблюдения за счет усиления продукции противовоспалительного ИЛ10 с сохранением эффекта в отдалённые сроки исследования (1,5 года).

✧ **Ключевые слова:** цилиарное тело; криохирургия; никелид титана; лабораторные животные.

BACKGROUND

Surgeries that decrease the production of intraocular fluid have been approved for the treatment of

the main types of refractory glaucoma to reduce intraocular pressure (IOP) and eliminate pain. Laser destruction of the ciliary body remains the principal

technique for the treatment of this disease but may lead to unsatisfactory IOP-lowering effects and hemorrhagic or inflammatory complications. In addition, laser exposure damages the ciliary body and causes uveitis leading to long-lasting and sometimes permanent effects [1]. Cryosurgical treatment using an autonomous cryoapplicator made of porous permeable titanium nickelide seems promising.

Ciliary body cryodestruction was proposed by Bietti in 1933 for the treatment of severe types of glaucoma. It is based on the ability to develop atrophic lesions in the ciliary body and thus achieve a lower anterior-chamber fluid production. Extremely low temperatures from $-130\text{ }^{\circ}\text{C}$ to $-190\text{ }^{\circ}\text{C}$ produce a more destructive effect and require less exposure time to achieve the desired results in comparison with moderately low temperatures from $-20\text{ }^{\circ}\text{C}$ to $-80\text{ }^{\circ}\text{C}$. Increased vascular permeability, local edema, rupture of small vessels producing small extravasates and microthrombosis, which completely disappear in a period of 1–2 weeks, are phenomena that are typical of cold injury that develop in tissues adjacent to areas destroyed by cold. However, as tissue temperature increases to normal levels, blood supply is restored. The development of a new generation of cryoapplicators by the Scientific Research Institute of Medical Substances and Implants with Shape Memory of Siberian Physical-Technical Institute at Tomsk State University has led to a substantial improvement in the quality of cryotherapy owing to a more precise localization of heat removal (cryotherapy) from the altered tissues. The developed applicators are autonomous, retain their cryogenic properties for long periods, provide an ultra-low “nitrogen” temperature on the surface of the object, and do not adhere to the tissues. Local cryotherapy is usually painless. Cryonecrotic lesions are characterized by a type of “biological inertness” and cause minimal perifocal reaction. In addition, they heal quickly without causing rough scarring, thus providing the best therapeutic effect [3]. The study of immunological reactivity indicators in eye injuries of various etiologies, including intraoperative injuries, may detect changes both at the systemic and local levels comprising the overproduction of proinflammatory cytokines, which correlates well with the severity of the posttraumatic period [2, 4]. These reveal multidirectional changes in the cytokine profile at the local and systemic levels, indicating a predominant role of local over systemic immunity in the injury of the immunologically privileged eye [6].

Therefore, the purpose of the study was to evaluate the level of locally produced pro- and anti-inflam-

matory cytokines in experimental animals after the direct and transconjunctival cryoablation of the ciliary body using an autonomous cryoapplicator made of permeable porous titanium nickelide.

MATERIALS AND METHODS

The concentration of cytokines (IL1 β , TNF α , IL-2, and IL-10) in the anterior-chamber fluid of experimental animals (21 Chinchilla rabbits of both sexes aged <2 years) was measured using enzyme immunoassay at 3 h, 7 h, 30 h, 3 months, and 1.5 years after the experimental destruction of the ciliary body using the autonomous cryoapplicator made of permeable porous titanium nickelide (group 1). The control group comprised 6 intact rabbits (group 2), and the comparison group comprised five rabbits in whom the traditional antihypertensive surgery had been performed (group 3). The enzyme immunoassay was conducted using testing systems using «Cytokine» LLC (St. Petersburg, Russian Federation) and «ASCENT» analyzer (Finland).

The operation was performed under general anesthesia. Anesthesia was achieved by an intramuscular injection of 0.4 ml of zoletil solution (dosage: 100 mg per 1 kg of body weight) and instillation of 0.4 % inokain. Subsequently, a conjunctival incision 5–6-mm long and 6–7 mm from the limbus was made with a dissection of Tenon’s capsule. The conjunctiva was separated toward the limbus and hemostasis was ensured. A square scleral flap measuring 4 \times 4 mm was formed from the superficial layers of the sclera, exposing the ciliary body. Direct cryodestruction of the ciliary body with an exposure of 35–42 s followed. Next, the scleral flap was returned back to its original position. The conjunctiva was sutured with a running stitch using monolithic thread made of titanium nickelide (TH-10) measuring 45 μ in diameter. Direct ciliary body cryodestruction according to the aforementioned methodology or a transconjunctival ciliary body cryoapplication with the specified exposure was performed in the neighboring sector of the eyeball. Anterior-chamber fluid sampling and conjunctival suture removal were also performed under general anesthesia.

The spine of the surgical instrument was a cryotransmitter in the form of a 2.5-mm-diameter rod made of permeable porous titanium nickelide with requisite pervasive porosity and pore structure. When the rod is placed in liquid nitrogen, the latter displaces the air contained in the pores, and within a minute the cryoapplicator is completely filled with refrigerant coolant like a sponge. The temperature of

a cryoapplicator's working part is $-200\text{ }^{\circ}\text{C}$; it has a high heat capacity, low thermal conductivity, and provides the conditions to momentarily produce a cryoeffect on biological tissues. The maximal effect of cryosurgical manipulation is possible for a period of 1–1.5 min when the porous titanium nickelide device, filled with liquid nitrogen, maintains a temperature close to the boiling point of nitrogen.

Statistical analysis included the calculation of the mean values including the calculation of the mean arithmetic (M), mean error (m), and the probability significance coefficient (P) using SPSS Statistics 17.0 for Microsoft computer program. Comparison of the average values of the two samples was performed using the Student's t -test. Differences were considered to be statistically significant (95 % CI, $p \leq 0.01$).

RESULTS AND DISCUSSION

The results of our study confirmed that the surgery itself produced certain changes in the local concentration of cytokines in all 3 groups of animals. In particular, we noted the inverse relationship of pro-inflammatory cytokine changes [IL1 β (statistically significant 1.5 times increase against the background values) and TNF α (statistically significant 3 times decrease)] at all studied time points. We found an increase in the IL-10 concentration, which was most pronounced on the 3rd day after surgery (2.4 times), and a reduction in IL-2 concentration

was observed later (7, 30 days). The detected pattern of changes made it necessary to compare these data from the main study group not only with the control group but also with a comparison group.

Therefore, the statistically significant increase of pro-inflammatory IL1 β concentrations at all follow-up time points after the direct destruction of the ciliary body using the cryoapplicator made of titanium nickelide was produced by the surgical intervention itself, whereas the concentration of TNF α significantly exceeded its level in both the control and comparison group at all follow-up time points (Table 1, Figure 1).

The increase of pro- (IL-2) and anti-inflammatory (IL-10) cytokines levels was more pronounced in the treated group in comparison with the TNF α levels (on average 4 times more compared to the comparison group; Table 1, Figure 1).

The evaluation of pro- and anti-inflammatory cytokine concentrations in the anterior-chamber fluid of the experimental animals in the long term after the destruction of the ciliary body using the cryoapplicator made of titanium nickelide was conducted at 3 months and 1.5 years. Three months after the intervention, it was found (see Table 1) that the concentration of IL-2 remained at a high level, and the concentration of pro-inflammatory IL1 β was the greatest among all follow-up time points and 3 times higher than its background level, whereas the concentrations of TNF α and IL-10 were within normal range. In the most long-term period of our study (1.5 years),

Table 1

The changes in cytokine concentration in the anterior chamber fluid of rabbit eyes after the destruction of the ciliary body using the cryoapplicator made of titanium nickelide ($M \pm m, p$)

Group	Ciliary body destroyed using the cryoapplicator made of titanium nickelide (group 1, n = 21)				
	IL1 β , pg/ml	TNF α , pg/ml	IL2, pg/ml	IL10, pg/ml	IL1 β + TNF α + IL2/IL10, conditional units
3 days	3.83 \pm 0.28*	7.34 \pm 1.03 *#	9.16 \pm 1.15*#	13.10 \pm 1.44*#	1.55 \pm 0.28#
7 days	3.35 \pm 0.49*	8.65 \pm 1.11 *#	16.40 \pm 1.43*#	14.23 \pm 1.27*#	2.01 \pm 0.34#
30 days	3.72 \pm 0.37 *	7.03 \pm 0.98 #	16.30 \pm 1.58*#	15.00 \pm 1.62*#	1.65 \pm 0.21
3 months	6.01 \pm 0.48*#	5.30 \pm 0.60#	10.50 \pm 0.96*#	6.82 \pm 0.70#	3.20 \pm 0.28*#
1.5 years	2.90 \pm 0.15#	6.48 \pm 0.55	17.90 \pm 1.45*	6.69 \pm 0.65#	4.08 \pm 0.50*#
Control group of intact rabbits (group 2, n = 6)					
Background	2.40 \pm 0.21	5.91 \pm 0.63	6.83 \pm 0.86	7.80 \pm 2.20	1.94 \pm 0.19
Comparison group of rabbits after the surgical intervention (group 3, n = 5)					
3 days	3.63 \pm 0.38*	1.59 \pm 0.24*	6.21 \pm 0.67	18.50 \pm 2.31*	0.97 \pm 0.14*
7 days	3.82 \pm 0.49*	2.55 \pm 0.34*	4.49 \pm 0.54*	10.68 \pm 2.47*	1.13 \pm 0.19*
30 days	3.69 \pm 0.45*	1.61 \pm 0.22*	5.04 \pm 0.48*	7.52 \pm 0.93*	1.51 \pm 0.21*
3 months	5.56 \pm 0.62*	7.11 \pm 0.85*	7.98 \pm 0.95	8.35 \pm 0.85	1.52 \pm 0.18
1.5 years	3.32 \pm 0.40	6.80 \pm 0.65	18.43 \pm 1.75*	9.80 \pm 0.85*	2.90 \pm 0.32

* — statistically significant compared to the values in the control group; # — statistically significant compared to the values in the comparison group

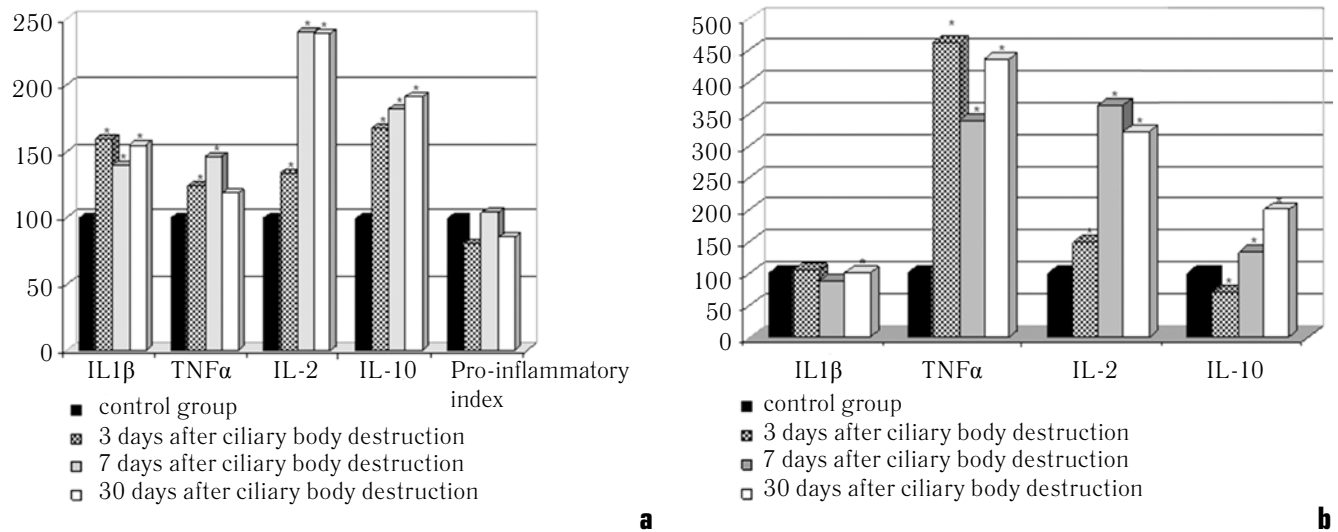


Fig. 1. The effect of ciliary body destruction using the cryoapplicator made of titanium nickelide on cytokine concentration in the anterior chamber fluid of rabbit eyes and their interrelationship during the follow-up period; a) % of the values in the control group, b) % of the values in the comparison group. * — The differences are statistically significant compared to the control group ($p < 0.01$, $p < 0.02$)

there was a reduction in the concentration of pro-inflammatory IL1β to the level of background values and normalization of TNFα and IL-10 concentrations; however, the level of IL-2 was still well above the reference value (on average 2 times higher) not only in the main experimental group but also in the control group (Table 1). Such changes in cytokine concentrations influence the value of the pro-inflammatory index, which was calculated as the ratio of the sum of pro-inflammatory cytokines (TNFα + IL1β + IL2) to an anti-inflammatory IL-10. It was shown that the pro-inflammatory index had initially been higher in the main experimental group, not because of the increase in the pro-inflammatory cytokine (IL1β and TNFα) concentrations but because of the high levels of IL-2, which plays an important regulatory role in the induction and maintenance of immune responses of both cellular and humoral types. The pro-inflammatory index value in animals with the destruction of the ciliary body using the cryoapplicator made of titanium nickelide on the 3rd and 7th day of the follow-up was higher than that in animals belonging to the control group ($p < 0.02$), whereas no statistically significant differences were found with the background values of the intact animals.

By the 30th day of observation, the shift of cytokine balance to the pro-inflammatory component had completely leveled and the values of the pro-inflammatory index corresponded to that in the control and comparison groups because of an increase in anti-inflammatory IL-10 (Table 1, Figure 2). In the most long-term period (3 months and 1.5 years), the value of the pro-inflammatory index was still

higher not only in the background control group but also in the appropriate comparison group. This was because of the increased production of IL-2 and the normalization of the levels of IL-10 in the main experimental group and cannot be regarded as the activation of the inflammatory process (Table 1, Figure 2).

After assessing the overall clinical condition of the rabbits used in the experiment, it is necessary to state that in a majority, the postoperative period pro-

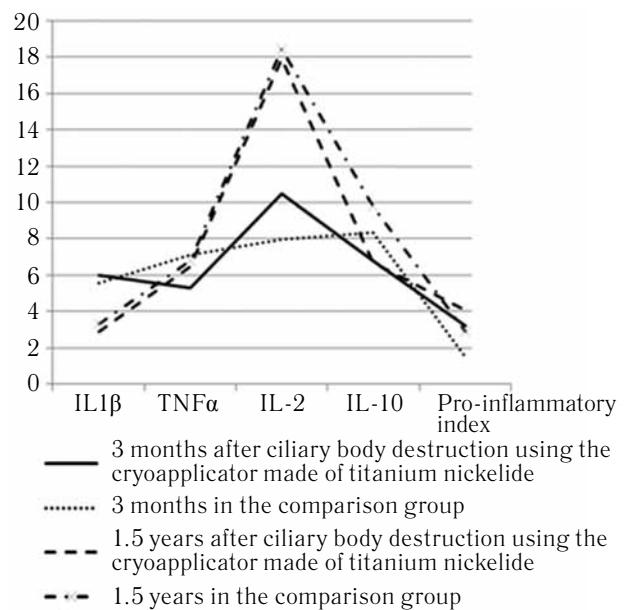


Fig. 2. The effect of ciliary body destruction using the cryoapplicator made of titanium nickelide on the ratio of pro-inflammatory to anti-inflammatory cytokine concentration in the anterior chamber fluid of rabbit eyes and their interrelationship during the follow-up period

ceeded without reactions, and the clinical manifestations of individual cases of viral infection (skin rashes around the eyes) in animals had fully resolved by the end of the follow-up period. The well-known cytokine high sensitivity to viral antigens [5] was the reason for excluding the samples of sick animals from the overall design of the study.

CONCLUSION

The assessment of the concentrations of pro-IL1 β , TNF α , and IL-2) and anti-inflammatory (IL-10) cytokines in the anterior chamber fluid of laboratory animals' eyes at 3, 7, and 30 days after the experimental destruction of the ciliary body using a cryoapplicator made of porous titanium nickelide demonstrated the predominance of pro-inflammatory cytokines in the short-term (3–7 days), and the normalization of the balance between pro- and anti-inflammatory cytokines in the long-term (30 days) follow-up period because of the increased production of anti-inflammatory IL-10, with the preservation of this effect in the longer-term follow-up period (1.5 years). These statistically significant changes in the concentration of the cytokines at the local level reflect the reactivity of the laboratory animals' organs to the surgical intervention in the ciliary body area and to experimental ciliary body destruction using the cryoapplicator made of titanium nickelide.

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