OPHTALMOLOGY

FEATURES OF THE LOCAL CYTOKINE PROFILE OF PATIENTS WITH BULLOUS KERATOPATHY BY USING PERSONALIZED THERAPY WITH CELLULAR TECHNOLOGIES

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ABSTRACT

Background. *Today we have active development of ophthalmic surgery, but the role of cytokines in the pathogenesis of bullous keratopathy remains insufficiently studied at present.*

The aim. *To study the dynamics of the local cytokine profile in bullous keratopathy by using personalized treatment with suspension of autologous blood mononuclears.*

Material and methods. *Two groups of patients with bullous keratopathy were formed: the main group (30 people, 30 eyes), who received an intrastromal injection of a suspension of autologous blood mononuclears and comparison (28 people, 28 eyes), who received a course of pharmacotherapy. The level of interleukins (IL) – IL-10, IL-4, IL-6, IL-1ß, transforming growth factor beta-2 (TGF-β2), tumor necrosis factor alpha (TNF-α) was determined in the lacrimal fluid of the patient's eye by enzyme immunoassay.*

Results. *All patients before treatment showed an increase of IL-1ß in the lacrimal fluid by 3.3 times, IL-6 – by 4.2 times, TNF-α – by 2.0 times ($p < 0.05$); an increase in the level of IL-4, IL-10 and TGF-β2 by 1.1 times ($p > 0.05$). There was a decrease in IL-1ß by 2.0 times, IL-6 – by 2.1 times and TNF-α – by 1.8 times, and an increase in IL-10 by 1.5 times, IL-4 – by 1.9 times, TGF-β2 – by 1.4 times ($p < 0.05$) in the main group after treatment, persisting for 12 months. There was a short-term decrease of IL-1ß by 1.7 times, IL-6 and TNF-α – by 1.2 times and an increase of IL-10, IL-4, TGF-β2 by 1.2 times ($p < 0.05$) in the comparison group which then reached the initial values.*

Conclusion. *There is an imbalance in the system of pro- and anti-inflammatory cytokines in bullous keratopathy in the lacrimal fluid. Injection of autologous mononuclear cells in the stroma of the cornea reduces the severity of the imbalance of the local cytokine system compared to the course of pharmacotherapy.*

Key words: *bullous keratopathy, cellular technologies, autologous mononuclear leukocytes, cytokines*

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ДИНАМИКА ЛОКАЛЬНОГО ЦИТОКИНОВОГО ПРОФИЛЯ ПРИ ЭНДОТЕЛИАЛЬНО-ЭПИТЕЛИАЛЬНОЙ ДИСТРОФИИ РОГОВИЦЫ НА ФОНЕ ПЕРСОНИФИЦИРОВАННОЙ ТЕРАПИИ С ИСПОЛЬЗОВАНИЕМ КЛЕТОЧНЫХ ТЕХНОЛОГИЙ

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РЕЗЮМЕ

Обоснование. *Активное развитие офтальмохирургии обусловливает увеличение числа пациентов с эндотелиально-эпителиальной дистрофией (ЭЭД) роговицы, роль цитокинов в патогенезе которой остаётся недостаточно изученной.*

Цель исследования: *изучить динамику локального цитокинового профиля при ЭЭД роговицы в условиях персонифицированного лечения с использованием суспензии аутологичных мононуклеаров крови.*

Материал и методы. *Сформировано две группы больных с ЭЭД роговицы: основная (30 человек, 30 глаз), получившая интрастромальное введение суспензии аутологичных мононуклеаров крови, и сравнения (28 человек, 28 глаз), получившая курс фармакотерапии. В слёзной жидкости (СЖ) больного глаза определяли уровень интерлейкинов (IL) – IL-10, IL-4, IL-6, IL-1β, трансформирующего фактора роста бета-2 (TGF-β2, transforming growth factor β2), фактора некроза опухоли альфа (TNF-α, tumor necrosis factor α) методом иммуноферментного анализа.*

Результаты. *У всех пациентов до лечения в СЖ выявлено повышение IL-1β в 3,3 раза, IL-6 – в 4,2 раза, TNF-α – в 2,0 раза ($p < 0,05$); увеличение уровней IL-4, IL-10 и TGF-β2 в 1,1 раза ($p > 0,05$). В основной группе после лечения отмечено снижение IL-1β в 2,0 раза, IL-6 – в 2,1 раза и TNF-α – в 1,8 раза; повышение IL-10 в 1,5 раза, IL-4 – в 1,9 раза, TGF-β2 – в 1,4 раза ($p < 0,05$), сохраняющееся в течение 12 мес. В группе сравнения отмечено краткосрочное снижение IL-1β в 1,7 раза, IL-6 и TNF-α – в 1,2 раза; увеличение уровней IL-10, IL-4, TGF-β2 в 1,2 раза ($p < 0,05$), которые затем достигли первоначальных значений.*

Заключение. *Выявлен дисбаланс в СЖ в системе про- и противовоспалительных цитокинов при ЭЭД роговицы. Интрастромальное введение аутологичных мононуклеров уменьшает выраженность дисбаланса локальной цитокиновой системы по сравнению с курсом фармакотерапии.*

Ключевые слова: *эндотелиально-эпителиальная дистрофия роговицы, клеточные технологии, аутологичные мононуклеарные лейкоциты, цитокины*

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RELEVANCE

Bullous keratopathy or endothelial-epithelial dystrophy of the cornea is a progressive disease of the cornea, the main manifestation of which is a chronic corneal edema and a pain syndrome. According to modern concepts [1–3], the pathogenesis is based on a decrease in the density of endothelial cells of the cornea, the cause of which may be injury, previous surgery or ocular inflammatory diseases. These changes lead to an increase in the permeability of the endothelial layer of the cornea, followed by hydration of the corneal stroma and keratocyte dystrophy [4, 5]. More and more data on the role of cytokines in the pathogenesis of bullous keratopathy have appeared in recent scientific publications [6-8]. However, these facts are currently fragmented, which makes it necessary to study in detail the local cytokine status in bullous keratopathy and develop new methods of treating this pathology of the cornea using cellular technologies.

THE AIM

To study the dynamics of the local cytokine profile in bullous keratopathy by using personalized treatment with suspension of autologous blood mononuclears.

MATERIAL AND METHODS

The presented clinical study was carried out at the Ophthalmology Clinic and the Central Research Laboratory of Siberian State Medical University (Tomsk). 58 patients with an established diagnosis of bullous keratopathy were under observation. The study was conducted in two stages. At the stage I of the study, the cytokine profile of the lacrimal fluid was studied in patients with bullous keratopathy. At the stage II the developed method of surgical treatment of bullous keratopathy using cellular technologies was tested in clinical practice and its effectiveness was assessed in comparison with pharmacotherapy. The approval of the clinical study was carried out by the local ethics committee of the Siberian State Medical University (registration number No. 5598, dated 06.11.2017). All patients signed a voluntary informed consent before the treatment.

The inclusion criteria: established diagnosis of bullous stage of bullous keratopathy (according to the classification of V.V. Volkov, M.M. Dronov, 1978), age from 18 to 85 years old. The exclusion criteria: general somatic diseases in the decompensation stage, mental illnesses, age under 18 years old, acute and chronic ocular inflammatory diseases in the exacerbation stage, monophthalm, pregnancy and lactation.

To study the local cytokine profile in bullous keratopathy in patients (58 patients, 58 eyes), the lacrimal fluid was taken from the inferior conjunctival fornix of the sore eye at the stage I of the clinical study. An automatic pipette was used to collect biological material. The lacrimal fluid was taken within 10 minutes from an inferior conjunctival fornix without prior stimulation. To study the samples obtained by enzyme immunoassay (ELISA), the following types of cytokines were selected: interleukins (IL) – IL-4, IL-10, IL-1β, IL-6, tumor necrosis factor α (TNF-α), transforming growth factor β2 (TGF-β2). Cytokine content was determined according to the attached instructions to the test systems: IL-10, IL-1β, IL-4, IL-6, TNF-α – Vector Best (Russia), TGF-β2 – Bender MedSystems (Austria). The ELISA results were recorded on a Uniplan vertical photometer (Russia) at a wavelength of 450 nm.

For the stage II of the clinical study, all patients (58 people), depending on the planned treatment, were divided into two groups: the main group and the comparison group. The main group of patients (30 people, 30 eyes) received personalized surgical treatment of bullous keratopathy by creating a local detachment of the Descemet's membrane due to the injection of sterile air into the posterior third of the cornea, followed by the injection of 0.3–0.4 ml of freshly isolated suspension of autologous mononuclear blood leukocytes into the area of the detached Descemet's membrane (patent No. 2674237 dated 05.12.2018). The surgical manipulation was performed once. In the postoperative period, patients received instillations of 0.3% solution of tobramycin 4 times a day for 7 days in order to prevent secondary infection.

Patients of the comparison group (28 people, 28 eyes) received a course of pharmacotherapy, including subconjunctival injections of 0.01% solution of riboflavin 1 time a day, instillations of 0.01% aqueous solution of vitamin B2, 5% dexapanthenol gel 4 times a day for 14 days.

The lacrimal fluid sampling from the conjunctival cavity of the sore eye in patients of both groups was carried out in the described way on the 3rd, 10th day from the onset of the treatment, as well as 1, 3, 6, 9 and 12 months after the treatment. The obtained lacrimal fluid samples were frozen at –20 °C, which made it possible to use them for three months.

The total duration of the study was 12 months.

Statistical processing of the results was carried out using the Statistica 13.3 program (StatSoft Inc., USA). After testing for normality of distribution using the Kolmogorov-Smirnov test the differences of dependent variables within the group were assessed using Wilcoxon's t-test, and the differences between the groups were assessed using Mann – Whitney U test. The results of this work are presented in the form $M \pm m$, where M is the sample mean, m is the error of the mean. Differences in indicators were considered statistically significant at the level of $p < 0.05$.

RESULTS

The gender and age distribution of patients was as follows. The main group included 30 patients, including 28 women and 2 men with an average age of 79.4 years, and in the comparison group – 28 people, including 27 women and 1 man ($p > 0.05$) with an average age of 77.7 years ($p > 0.05$).

According to the results of the study of the local cytokine profile in patients with bullous keratopathy, an increase in the content of proinflammatory cytokines (IL-1ß, IL-6, TNF-α) was detected in the samples of the the lacrimal fluid before the treatment. Thus, the baseline of IL-1ß in both

groups was increased by 3.3 times compared to the normal value ($p < 0.05$) [9], the level of IL-6 in both groups was increased by 4.2 times ($p < 0.05$) [10], the TNF-α index in both groups was 2.0 times higher than the normal value ($p < 0.05$) [11] (Tables 1, 2). There were no statistically significant differences in the indicators of these cytokines in patients of the main group and the comparison group before the treatment ($p > 0.05$) (Tables 1, 2).

The baseline level (before treatment) of IL-10, IL-4, TGF-β2 in the lacrimal fluid of the sore eye in all patients before the treatment was 1.1 times higher than the normal value [10, 12, 13], which, however, was not statistically significant ($p > 0.05$) (Tables 1, 2).

According to the results of the stage II of the study, on the 3rd day from the onset of the treatment, patients of both groups showed a slight, not statistically significant, decrease in the level of IL-1ß in the samples of the lacrimal fluid of the sore eye compared with the baseline ($p > 0.05$) (Tables 1, 2). On the 10th day after treatment with a new surgical method with using cellular technologies in patients of the main group, the level of this cytokine in the lacrimal fluid of the sore eye decreased by 1.7 times ($p < 0.05$) from the baseline (Table 1), while in patients of the comparison group who received conservative treatment – by 1.3 times ($p < 0.05$) (Table 2). 1 month after intrastromal injection of a suspension of autologous blood mononuclears the maximum decrease in the level of IL-1ß in the lacrimal fluid in patients of the main group during the entire observation period was 2.0 times compared with the baseline ($p < 0.05$) (Table 1), in patients of the comparison group – by 1.7 times from the baseline

TABLE 1

THE DYNAMICS OF CYTOKINE LEVEL IN LACRIMAL FLUID IN PATIENTS OF THE MAIN GROUP BEFORE AND AFTER THE TREATMENT, PG/ML (M ± m)

Follow-up period	Cytokines					
	IL-1β	IL-6	TNF-α	IL-4	IL-10	TGF-β2
Standard	18.2 ± 3.5	125.3 ± 11.7	252.6 ± 11.1	26.3 ± 7.4	142.4 ± 5.0	168.5 ± 25.3
Before the treatment	59.3 ± 9.5	523.6 ± 16.8	497.6 ± 20.5	29.8 ± 2.7	156.4 ± 3.3	184.4 ± 8.3
3rd day	56.4 ± 7.8*	420.3 ± 18.6*	354.8 ± 28.8*	69.4 ± 4.8*	275.4 ± 5.2*	207.6 ± 8.9
10th day	34.2 ± 5.2*	336.8 ± 24.6*	320.4 ± 23.7*	45.4 ± 3.8*	294.3 ± 5.6*	205.8 ± 11.2
1 m	29.8 ± 3.3*	305.4 ± 23.3*	294.8 ± 19.2*	42.4 ± 1.6*	286.9 ± 4.4*	258.1 ± 16.1*
3 m	33.4 ± 4.9*	254.3 ± 24.7*	284.3 ± 18.94*	39.5 ± 1.4*	296.3 ± 3.7*	245.4 ± 14.7*
6 m	32.5 ± 3.7*	246.8 ± 25.3*	275.3 ± 17.5*	40.4 ± 1.5*	244.8 ± 4.0*	179.5 ± 7.9
9 m	34.6 ± 3.8*	236.7 ± 23.8*	279.6 ± 15.9*	44.7 ± 1.4*	232.1 ± 2.2*	180.3 ± 8.4
12 m	34.9 ± 3.1*	243.8 ± 21.3*	278.4 ± 13.3*	43.2 ± 1.3*	230.3 ± 1.8*	182.4 ± 8.9

Note. * – the level of statistical significance of the differences compared to the baseline ($p < 0.05$).

TABLE 2

THE DYNAMICS OF CYTOKINE LEVEL IN LACRIMAL FLUID IN PATIENTS OF THE COMPARISON GROUP BEFORE AND AFTER THE TREATMENT PG/ML (M ± m)

Follow-up period	Cytokines					
	IL-1β	IL-6	TNF-α	IL-4	IL-10	TGF-β2
Standard	18.2 ± 3.5	125.3 ± 11.7	252.6 ± 11.1	26.3 ± 7.4	142.4 ± 5.0	168.5 ± 25.3
Before the treatment	58.4 ± 7.9	519.6 ± 12.2	502.4 ± 10.5	30.2 ± ,3.0	158.4 ± 4.3	179.7 ± 6.1
3rd day	57.2 ± 7.1	495.7 ± 10.1*	482.3 ± 12.0	33.5 ± 3.4	162.5 ± 4.3	189.3 ± 9.0
10th day	45.3 ± 6.7*	478.5 ± 8.0	435.3 ± 17.7*	35.6 ± 3.7*	189.4 ± 6.8*	195.4 ± 9.6
1 m	35.6 ± 6.1*	450.6 ± 13.5*	472.8 ± 18.4	31.3 ± 3.3	162.4 ± 5.1	215.3 ± 8.5*
3 m	39.7 ± 4.9*	487.5 ± 17.6	485.3 ± 15.9	27.8 ± 2.8	154.3 ± 5.4	188.3 ± 7.6
6 m	49.3 ± 4.7*	501.7 ± 13.4	492.3 ± 10.2	26.9 ± 2.7	157.5 ± 6.5	175.2 ± 8.9
9 m	53.4 ± 4.5	510.7 ± 11.2	489.6 ± 7.4	27.5 ± 3.0	156.1 ± 5.6	172.8 ± 9.4
12 m	54.7 ± 5.9	517.7 ± 10.4	485.3 ± 7.3	26.4 ± 2.3	149.8 ± 5.9	184.3 ± 8.2

Note. * – the level of statistical significance of the differences compared to the baseline ($p < 0.05$).

($p < 0.05$) (Table 2). In 3 months the level of IL-1ß in the lacrimal fluid of the sore eye in the patients of the main group increased slightly to 33.4 pg/ml ($p > 0.05$), but subsequently remained stable throughout the follow–up (Table 1). The level of this cytokine in the lacrimal fluid of the sore eye in patients of the comparison group gradually increased during observation and reached the baseline before treatment 12 months after the course of pharmacotherapy (Table 2).

On the 3rd day after the presented surgical treatment the level of IL-6 in the lacrimal fluid of the sore eye in the main group of patients decreased by 1.3 times compared to the baseline ($p < 0.05$) (Table 1), while in patients of the comparison group this indicator decreased by 1.1 times ($p > 0.05$) (Table 2). 1 month after the treatment the level of this cytokine in the main group decreased by 1.7 times from the baseline ($p < 0.05$) (Table. 1), in the comparison group – only 1.2 times ($p < 0.05$) (Table 2). 3 months after intrastromal injection of a suspension of autologous blood mononuclears the content of IL-6 in the lacrimal fluid of the sore eye in patients of the main group decreased by 2.1 times ($p < 0.05$) from the baseline, and subsequently its stabilization was noted throughout the entire follow-up period (Table 1). The level of this cytokine in patients of the comparison group began to gradually increase 3 months after the course of pharmacotherapy and reached the baseline after 12 months (Table 2).

The level of TNF-α in the lacrimal fluid of the sore eye in patients of the main group on the 3rd day after the injection of a suspension of autologous blood mononuclears into the stroma of the cornea decreased by 1.4 times ($p < 0.05$) from the baseline (Table. 1), while in the comparison group against the background of conservative treatment – only 1.1 times ($p > 0.05$) (Table 2). The TNF-α level in patients of the main group decreased 1.6 times from the baseline on the 10th day after the onset of the treatment ($p < 0.05$) (Table 1), in the comparison group – by 1.2 times ($p < 0.05$) (Table 2). The level of TNF-α in the lacrimal fluid in the main group continued to decrease during further follow-up and reached the minimum value by the 6th month of follow-up (Table 1). In the comparison group, on the contrary, a gradual increase in the content of this cytokine was noted (Table 2).

The content of IL-4 in the lacrimal fluid in patients of the main group with bullous keratopathy on the 3rd day after the described surgical treatment increased 2.3 times ($p < 0.05$) from the baseline (Table 1), while in patients of the comparison group who received conservative treatment it was only 1.1 times ($p > 0.05$) (Table 2). On the 10th day after intrastromal injection of a suspension of autologous mononuclears in patients of the main group the level of the studied cytokine exceeded the baseline by 1.5 times ($p < 0.05$) (Table 1), in patients of the comparison group – by 1.2 times ($p < 0.05$) (Table 2). 1 month after treatment the content of IL-4 in the lacrimal fluid of the sore eye in patients of the main group exceeded the baseline by 1.4 times ($p < 0.05$). At the same time, this indicator remained relatively stable throughout the entire follow-up period (12 months) (Table 1). 1 month after the course of pharmacotherapy the content of IL-4 in the lacrimal fluid of the sore eye in patients of the comparison group did not significantly differ from that before the treatment, and subsequently, its gradual decrease was observed within 12 months (Table 2).

The level of IL-10 in the lacrimal fluid of the sore eye in patients of the main group on the 3rd day after the injection of a suspension of autologous mononuclears into the stroma of the cornea increased by 1.7 times from the baseline ($p < 0.05$) (Table 1). There was no statistically significant change in this cytokine in patients of the comparison group (Table 2). On the 10th day after surgical treatment with using cellular technologies the level of IL-10 in the lacrimal fluid in patients of the main group increased by 1.8 times from the baseline ($p < 0.05$) (Table 1), in the comparison group – by 1.2 times ($p < 0.05$) (Table 2). After 1 month after surgical treatment the level of this cytokine in the lacrimal fluid of the sore eye in patients of the main group exceeded the baseline by 1.7 times ($p < 0.05$), and after 3 months – by 1.9 times ($p < 0.05$). Stabilization of the level of IL-10 in the lacrimal fluid of the sore eye was revealed during further follow-up (up to 12 months) after a slight, non-statistically significant decrease ($p > 0.05$) compared with the indicator after 6 months (Table 1). 10 days after the onset of pharmacotherapy a statistically significant increase (1.2 times; $p < 0.05$) in the level of this cytokine in the lacrimal fluid of the sore eye in the comparison group was noted, and by the end of observation (12 months) its content in the lacrimal fluid corresponded to that before treatment (Table 2).

The level of TGF-β2 in the lacrimal fluid of the sore eye on the 3rd day after treatment in patients of both groups increased only 1.1 times from the baseline (Tables 1, 2), which is not statistically significant ($p > 0.05$). This tendency persisted in both groups (Tables 1, 2) on the 10th day after treatment. After 1 month the level of TGF-β2 in the lacrimal fluid of the sore eye in patients of the main group increased by 1.4 times ($p < 0.05$) from the baseline (Table 1), in the comparison group – by 1.2 times ($p < 0.05$) (Table 2). Further follow-up revealed a gradual decrease in the level of TGF-β2 in both groups, which reached the baseline by 12 months after the treatment (Tables 1, 2).

DISCUSSION

The analysis of the obtained results allows to detect a statistically significant increase in the lacrimal fluid of the sore eye of proinflammatory cytokines IL-1ß, IL-6, TNF-α in all patients with bullous keratopathy before the treatment, which indicates a significant role of inflammation in the pathogenesis of this disease. Thus, IL-1ß is a cytokine that induces inflammation at the local level and increases the chemotaxis of effector cells to the pathological focus [14]. IL-6 also contributes to the activation of inflammation against the background of a decrease in the control function of excessive inflammatory response [15], and TNF-α is one of the most significant proinflammatory cytokines and participates in the pathogenesis of many immuno-inflammatory diseases. TNF-α increases the functional activity of leukocytes and macrophages, enhancing phagocytosis and cytokine synthesis [15].

At the same time, a slight increase in the content of cytokines such as IL-10 and -4, TGF-β2, which is not statistically significant ($p > 0.05$), was detected in the lacrimal fluid of the sore eye in all patients with bullous keratopathy before the treatment. These biologically active substances are multifunctional cytokines that regulate a large number of biological processes, such as migration, proliferation and apoptosis of various types of cells, and have anti-inflammatory and immunosuppressive effects [14, 15, 16]. The features of the local cytokine profile revealed during the study in bullous keratopathy indicate the presence of an imbalance in the system of pro- and anti-inflammatory cytokines, the severity of which to a certain extent may determine the severity of the course of dystrophic lesions of the cornea.

According to the results of the stage II of the study, the injection of a suspension of autologous blood mononuclears into the stroma of the cornea during bullous keratopathy as a source of cytokines contributes to the gradual restoration (within three months) of the level of the studied cytokines in the lacrimal fluid of the sore eye, reducing the existing imbalance. This, in turn, provides relief of inflammation with stabilization of the pathological process in the dystrophically altered cornea.

The course of conservative treatment carried out in patients of the comparison group, according to the data obtained, provides only a short-term reduction (within 1 month) of the imbalance in the local cytokine system in bullous keratopathy with subsequent regression.

CONCLUSIONS

There is an imbalance in the system of pro- and anti-inflammatory cytokines in the pathogenesis of bullous keratopathy. And this imbalance to a certain extent determines the severity of the disease.

The new personalized surgical method of treatment of bullous keratopathy with using cellular technologies allows to reduce the severity of the imbalance of the local cytokine system in comparison with the course of pharmacotherapy, ensuring the stabilization of the pathological process.

Conflict of interest

The authors of this article declare the absence of a conflict of interest.

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