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DYNAMICS OF THE INFLAMMATORY RESPONSE AND NEOVASCULARIZATION FOLLOWING STEM CELL APPLICATION IN THE TREATMENT OF CORNEAL ALKALI BURNS

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An imbalance between pro- and anti-angiogenic regulators resulting from reactive inflammation leads to corneal neovascularisation and a loss of corneal transparency. An experimental model of reactive inflammation and angiogenesis in the corneal tissues following an alkali burn was established in 30 white rabbits. Alkaline corneal burns in the acute phase were accompanied by an intensification of cellular and humoral factors of immune defence, and excessive production of pro-inflammatory cytokines, which are potential stimulators of pathological angiogenesis – neovascularisation, fibrosis and loss of transparency. The application to the burn wound of mesenchymal stem cells modified with monoclonal antibodies against CD73, CD90, CD105, CD34 and CD45, is accompanied by a statistically significant modulation of both the cellular and humoral immune responses, with a statistically significant acceleration of the inflammatory process, which reduces the activity of angiogenesis and neovascularisation and ensures the transparency of the cornea.

Key words: experimental alkaline corneal burn, inflammation, cellular and humoral immunity, neovascularisation, stem cells.

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ДИНАМІКА ЗАПАЛЬНОЇ РЕАКЦІЇ ТА НЕОВАСКУЛЯРИЗАЦІЇ ПІД ВПЛИВОМ АПЛІКАЦІЇ СТОVBУРОВИХ КЛІТИН ПРИ ЛУЖНОМУ ОПІКУ РОГІВКИ ОКА

Порушення балансу між про- та антиангіогенними регуляторами внаслідок реактивного запалення приводить до неоваскуляризації рогівки та втрати її прозорості. Експериментальну модель реактивного запалення та ангіогенезу у тканинах рогівки ока при її лужному опіку відтворено на 30 білих кролях. Лужний опік рогівки в гострому періоді супроводжувався інтенсифікацією клітинних та гуморальних факторів імунного захисту, надмірним продукуванням прозапальних цитокінів, які є потенціальними стимуляторами розвитку патологічного ангіогенезу – неоваскуляризації, фіброзу та втрати прозорості. Аплікації на опікову рану мезенхімальних стовбурових клітин, модифікованих моноклональними антитілами проти CD73, CD90, CD105, CD34 та CD45, супроводжується достовірно вираженою модуляцією як клітинної, так і гуморальної імунної відповіді, з достовірно прискореним перебігом запального процесу, що знижує активність ангіогенезу та неоваскуляризації і забезпечує її прозорість.

Ключові слова: експериментальний лужний опік рогівки, запалення, клітинний і гуморальний імунітет, неоваскуляризація, стовбурові клітини.

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According to WHO experts, chemical burns of the cornea are a widespread medical and social problem worldwide, as their incidence accounts for more than 35 % of all cases of corneal injuries [17] and they often result in blindness [9]. An important condition for normal light transmission through the cornea [6] is its avascularity, while burn injury to corneal tissues causes an immediate inflammatory response with migration of neutrophils, which perform a phagocytic function by clearing the burn wound of cellular debris; however, their excessive activity and release of proteolytic enzymes and reactive oxygen species may aggravate damage to the collagen structure of the corneal stroma and stimulate the immune-inflammatory response [16]. Studies by Smith et al. (2018) demonstrated that corneal epithelial injury in rabbits leads to rapid release of pro-inflammatory cytokines such as IL-1 β , TNF- α , and IL-6, which contributes to edema and degradation of the corneal stroma and complicates regeneration processes [15].

At present, most researchers consider that the development of a torpid immune-inflammatory process in corneal alkali burn leads to stimulation of angiogenesis and corneal neovascularization, which results in corneal opacity and deterioration of its optical properties [1, 14].

Protocol-based treatment methods for chemical corneal burns using antienzymatic, antioxidant, cytomembrane-protective, and anti-inflammatory agents, including glucocorticosteroids, have proven insufficiently effective or limited in terms of preventing chronicity of the process and development of leukoma. This clinical situation prompts the development of more biologically oriented restorative treatment methods, with active investigation into the potential of stem cells for correction of corneal burn injury and its complications, particularly for modulation of the immune-inflammatory process [12, 19], reduction of inflammation, and promotion of long-term tissue regeneration, in contrast to dexamethasone, which had a temporary effect [5].

Promising results have been obtained in studies using MSCs with multipotent properties for the treatment of chemical corneal burns have shown promising results [12], demonstrating that MSC treatment improves healing of the burn wound and restores the corneal surface through MSC differentiation. However, other studies have demonstrated that the therapeutic effect is achieved through suppression of inflammation and angiogenesis rather than through differentiation of stem cells [8].

Thus, the current absence of a unified concept regarding the sanogenetic and pathogenetic mechanisms of the restorative effect of MSC application in corneal alkali burns substantiates the feasibility of studying the mechanisms of their therapeutic effect and developing a method for their application.

The purpose of the study was to investigate the dynamics of reactive inflammation and angiogenesis in the tissues of the cornea following an alkali burn in an experimental setting under the influence of applications of pluripotent mesenchymal stem cells in Corneregel.

Materials and methods. The experiments were performed using a standardized experimental model [16], in which corneal injury was induced in rabbit eyes under local epibulbar anesthesia with 0.5 % Alcaine solution and retrobulbar anesthesia with 2 % lidocaine solution. Burn injury was induced by applying a 5-mm-diameter filter paper soaked in 1 N sodium hydroxide (NaOH) solution to the cornea for 30 seconds.

The conditions for housing and use of animals in the experiments complied with the requirements of current regulatory documents in accordance with Directive No. 2010/63/EU "On the protection of animals used for scientific purposes" and the provisions of the "European Convention for the Protection of Animals used for Experimental and Other Scientific Purposes" (Strasbourg, 1986).

The experimental rabbits were divided into 3 groups: group I (control) – intact animals (10 animals); group II – animals in which a chemical corneal burn was modeled (10 rabbits) and no intervention was performed during the course of the burn injury; group III – 10 animals in which a chemical corneal burn was modeled and the process was corrected using applications of mesenchymal stem cells in the preparation "Corneregel" (Dexpanthenol, Pharma Fabrik GmbH, Germany).

The source of mesenchymal stem cells (MSCs) was umbilical cord tissue, from which a tissue suspension was prepared according to the generally accepted method [2] under aseptic conditions and subjected to enzymatic treatment with 0.1 % collagenase I solution (Sigma-Aldrich, USA) dissolved in Advanced DMEM/F12 medium (Gibco, USA). The cell suspension was centrifuged, and the pellet was resuspended in culture medium with 10 %

fetal bovine serum (FBS). Isolated MSCs were cultured in an incubator at 37 °C with a CO₂ concentration of 5 %. Cell passaging was performed by trypsinization with TrypLE enzyme (Gibco, USA) after reaching 90–100 % confluence. Immunophenotyping of the obtained MSCs was performed by flow cytometry (BD Accuri™ C6 Plus Personal, Becton Dickinson, USA) using mouse monoclonal antibodies against CD73, CD90, CD105, CD34, and CD45 (Invitrogen, USA). MSCs were cultured using Advanced DMEM/F12 medium (Gibco, USA) supplemented with 10 % FBS, 1 % L-glutamine-penicillin-streptomycin solution (Sigma, USA), and heparin (240 µg/L) (Sigma, USA). MSCs at passage 10 were used in the experiment. A cell suspension containing 4.35 million MSCs in 1 mL of physiological sodium chloride solution per 1 g of Corneregel was prepared.

For biochemical studies, blood was collected from the marginal ear vein of the rabbit on days 1, 7, 14, and 21 after induction of the corneal burn injury, which corresponded to the stages of the burn process in corneal tissue: primary necrosis (day 1), acute vascular disorders and edema of ocular tissues (days 1–7); secondary necrosis and acute keratouveitis (days 7–14); trophic disorders and corneal ulceration (days 14–21); reparative regeneration (after day 21). On day 21 of the experiment, under thiopental sodium anesthesia (25 mg/kg), the rabbits were euthanized by air embolism.

The activity of the inflammatory process was assessed by the dynamics of cellular and humoral immunity parameters. Lymphocyte typing was performed by assessing the interaction of monoclonal antibodies labeled with a fluorescent marker with surface lymphocyte antigens [3]. Each tube contained 20 µL of antibodies against CD4+, CD8+, CD22+ and EDTA. The samples were analyzed using an Epics-XL flow cytometer (Beckman Coulter, USA). Serum immunoglobulin classes A, M, and G were studied by enzyme-linked immunosorbent assay using "GeneTex" reagent kits (USA) on a STAT-FAX enzyme immunoassay analyzer and expressed as g/L.

The level of circulating immune complexes (CICs) in blood serum was determined by precipitation with polyethylene glycol-6000 solution according to the standard method and photometrically measured using a KFK-3 at $\lambda=450$ nm. The number of CICs was calculated using the formula: CIC conventional units = $(E_d - E_c) \times 1000$, where E_d is the extinction of the polyethylene glycol tube contents; E_c is the extinction of the borate tube contents.

The state of the systemic inflammatory process was assessed based on TNF- α levels (pg/mL) and studied by enzyme-linked immunosorbent assay using "ELISA Kit for Rabbits Usen, Life Science Inc." reagent kits (USA) on a STAT-FAX enzyme immunoassay analyzer. Functional and bactericidal activity of neutrophils was determined using the nitroblue tetrazolium reduction test (NBT test) [11].

The degree of corneal neovascularization was studied in a rabbit corneal alkali burn model according to the Efron scale on days 7–14–21 after burn injury. The degree of corneal neovascularization was assessed according to the severity of bulbar redness (from 0=normal to 4=severe) using an ophthalmoscope.

Statistical processing of the obtained results was performed using the “Statistica 10.0” statistical software package and “Microsoft Excel-2013” using parametric and non-parametric evaluation methods with Student’s t-test and Mann–Whitney U-test. The difference between values was considered significant at $P < 0.05$.

Results of the study. The following clinical criteria were selected to characterize the course of the corneal alkali burn process: conjunctival hyperemia; corneal edema; corneal epithelial damage; persistent epithelial-stromal defects (PESD); the degree of corneal neovascularization was assessed according to the severity of bulbar redness using the Efron scale (from 0=normal to 4=severe). According to these criteria, it was established that one day after induction of the alkali burn, conjunctival hyperemia and corneal epithelial damage were observed in all experimental rabbits, as indicated by fluorescein staining. PESDs were absent at this observation period.

On day 7 after the corneal alkali burn in the control group (without pharmacological correction of the burn), severe conjunctival hyperemia was observed in 60 % of experimental animals and moderate hyperemia in 40 %. Corneal edema, as a manifestation of dystrophic changes, was more pronounced on day 7 of the experiment and gradually decreased by day 14. This pattern of the process determined the corresponding frequency of epithelial damage and formation of PESDs. At the same time, in experimental group III, in which applications of stem cells modified with monoclonal antibodies against CD73, CD90, CD105, CD34, and CD45 were applied on the burn wound, a significant reduction in clinical manifestations of the inflammatory process in the eye was observed. The frequency of symptoms such as conjunctival hyperemia, corneal edema, and epithelial damage significantly decreased, accompanied by a significant reduction in the number of persistent epithelial-stromal defects (PESDs) of the cornea. Regarding manifestations of neovascularization, it can be stated that during the corneal injury phase (1 day after the burn), it was manifested only by hyperemia of existing microvessels, while initial manifestations of angiogenesis and neovascularization appeared 7–14 days after the burn and were most pronounced after three weeks after the burn. It should be noted that the incidence and extent of angiogenesis in rabbits treated for the burn wound with applications of stem cells in Corneregel were significantly lower. Therefore, it can be concluded that in the acute period, animals with corneal alkali burns develop clinical signs of a reactive inflammatory process with dystrophic-necrotic changes in the cornea,

development of PESDs, and stimulation of angiogenesis in the cornea, which resulted in loss of its transparency. In the group of experimental animals in which applications of stem cells in Corneregel were applied to the burn wound, a significant reduction in manifestations of the inflammatory process and corneal neovascularization was observed.

The dynamics of changes in nonspecific resistance of the organism and the immune system in animals with corneal alkali burns treated with topical applications of MSCs in Corneregel was assessed by comparative analysis with those of untreated animals (Table 1). A significant increase in the sNBT-test parameters in rabbits with corneal burns was detected as early as the first day of the experiment; the content of active neutrophils in the blood increased 1.8-fold compared with the control ($p < 0.001$). The induced NBT-test parameters also increased, although to a lesser extent, but significantly (1.5-fold), therefore the reserve parameter decreased 1.2-fold, which was significantly lower than in the control. A decrease in the reserve parameter reflects a decrease in the functional reserves of neutrophils characteristic of inflammation. This is also indicated by a significant decrease in the metabolic activation coefficient of neutrophils (1.5-fold compared with healthy animals). Further observation of the dynamics of functional neutrophil activity in the untreated animals demonstrated a significant decrease in sNBT-test and iNBT-test parameters to 140 % and 115 %, respectively, on day 7 after burn injury, with a simultaneous decrease in the reserve capacity by 24 % and the reserve coefficient by 66 % compared with normal values. On days 14–21, the s-NBT-test parameter remained 1.5- to 1.3-fold higher than normal against a decrease in the i-NBT-test and further decrease in the reserve parameter (1.4-fold) and stimulation coefficient (2.3-fold), which did not recover by day 21 of the experiment.

When comparing these parameters of functional neutrophil activity in rabbits treated with applications of Corneregel and stem cells to the corneal burn wound, significant differences were observed. As early on days 7–14 of treatment, a significant decrease in functional neutrophil activity compared with untreated animals was observed, while following stimulation with pyrogenal, the number of neutrophils in the iNBT-test increased to a significantly lesser degree, which was accompanied by a decrease in the reserve parameter and activation coefficient. It should be noted that by days 21–28 of observation, functional neutrophil activity according to the studied parameters in treated animals was significantly better and reached the level of intact rabbits. It should also be noted that the decrease in the reserve parameter and the activation coefficient occurs in phases and dynamically depends on the stage and activity of the process, is characteristic of inflammation, and reflects a decrease in the functional reserves of neutrophils.

Table 1

Dynamics of nonspecific resistance parameters in experimental animals with corneal alkali burns under the influence of stem cell applications in Corneregel, M±m

Groups of animals		s-NBT-test, %	i-NBT-test, %	RP	C _{active}
Intact group (n=10)		15.5±0.92	25.9±0.79	1.7±0.06	0.4±0.02
Untreated burn, (n=10)	Day 1	27.6±0.89 P ₁ <0.001	38.6±0.61 P ₁ <0.001	1.4±0.06 P ₁ <0.05	0.3±0.03 P ₁ <0.01
MSC+Corneregel, (n=10)	Day 1	28.9±0.83 P ₂ ≥0.001	39.9±0.54 P ₂ ≥0.001	1.4±0.05 P ₂ ≥0.05	0.3±0.03 P ₂ ≥0.01
Untreated burn, (n=10)	Day 7	21.7±0.74 P ₁ <0.001	29.7±0.72 P ₁ <0.01	1.5±0.08 P ₁ <0.001	0.3±0.03 P ₁ <0.001
MSC+Corneregel, (n=10)	Day 7	17.3±0.71 P ₂ <0.001	27.4±0.72 P ₂ <0.01	1.5±0.08 P ₂ ≥0.05	0.4±0.03 P ₂ <0.05
Untreated burn, (n=10)	Day 14	22.94±0.37 P ₁ <0.001	26.9±0.62 P ₁ <0.05	1.2±0.04 P ₁ <0.001	0.2±0.03 P ₁ <0.001
MSC+Corneregel, (n=10)	Day 14	16.9±0.37 P ₂ <0.001	26.5±0.62 P ₂ ≥0.05	1.5±0.04 P ₂ <0.001	0.4±0.03 P ₂ <0.001
Untreated burn, (n=10)	Day 21	20.7±0.39 P ₁ <0.001	32.7±0.76 P ₁ <0.001	1.5±0.06 P ₁ >0.05	0.3±0.03 P ₁ >0.05
MSC+Corneregel, (n=10)	Day 21	16.1±0.39 P ₂ <0.001	26.2±0.76 P ₂ <0.001	1.6±0.06 P ₂ ≥0.05	0.4±0.03 P ₂ ≥0.05

Notes: p₁ – significance of the difference between the parameter in intact animals and the parameter in animals with untreated corneal burns; p₂ – significance of the difference between the parameter in untreated and treated animals with corneal burns.

Analysis of cellular and humoral immunity parameters in rabbits with corneal alkali burns indicated its activation at all stages of the study (Table 2). As early as the first day after the corneal burn, the CD4⁺ parameter increased by 26 % compared with healthy animals, while CD8⁺ increased by 11 %, resulting in a 1.2-fold increase in the immunoregulatory index. Similar changes were observed by day 7 after the burn, accompanied by a further increase in the CD4⁺/CD8⁺ ratio to 1.9 units. By day 14, a gradual decrease in the CD4⁺ parameter was observed (by 21 % compared with normal values) against an increase in CD8⁺ (by 11 %), resulting in a decrease in the immunoregulatory index to 1.64 and its actual normalization by day 21. Regarding the dynamics of B-lymphocyte content (CD22⁺), their

number increased slightly during the acute period of the burn process, while during the period of burn tissue repair it was significantly below normal. Their functional activity also increased accordingly: the concentrations of IgG, IgM, and IgA in the blood serum of animals with chemical corneal burns 24 hours after injury increased 1.3–1.7-fold compared with those in healthy animals, and a further increase by day 7 (2-fold above normal) was observed, followed by a gradual decrease by day 21. The obtained results of the dynamics of humoral immunity parameters in animals with chemical corneal burns indicate that at the initial stages of the pathological process, active mobilization of immunoglobulins from the bloodstream occurs, followed by suppression of their secretion at the systemic level.

Table 2

Dynamics of parameters of the cellular and humoral components of immunity in animals with corneal alkali burns under the influence of stem cell applications, M±m

Animal group		CD4 ⁺ /CD8 ⁺	CD22 ⁺ , %	Ig G, g/L	CICs, conventional units	TNF-α, pg/mL
Intact group (n=10)		1.51±0.06	12.18±0.23	3.66±0.06	292.3±7.6	0.672±0.052
Chemical corneal burn (n=20)	Day 1. untreated	1.73±0.06 P ₁ <0.01	13.63±0.32 p ₁ <0.001	5.92±0.12 p ₁ <0.001	316.7±5.9 p ₁ <0.001	0.924±0.043 P ₁ <0.001
	Day 1. MSCs + Corneregel	1.74±0.06 P ₂ >0.05	13.76±0.25 p ₂ >0.05	5.96±0.11 p ₂ >0.05	366.2±5.6 p ₂ <0.05	0.892±0.046 P ₂ >0.05
	Day 7. untreated	1.87±0.05 P ₁ <0.001	14.36±0.42 p ₁ <0.001	6.16±0.11 p ₁ <0.001	432.7±5.4 p ₁ <0.001	1.142±0.061 P ₁ <0.001
	Day 7. MSCs + Corneregel	1.72±0.05 P ₂ >0.05	14.84±0.36 p ₂ >0.05	6.47±0.08 p ₂ <0.05	485.8±5.1 p ₂ <0.05	1.043±0.052 P ₂ >0.05
	Day 14. untreated	1.64±0.06 P ₁ >0.05	10.73±0.35 p ₁ <0.001	5.32±0.09 p ₁ <0.001	532.5±6.3 p ₁ <0.001	0.983±0.044 P ₁ <0.001
	Day 14. MSCs + Corneregel	1.56±0.06 P ₂ >0.05	12.63±0.34 p ₂ <0.05	5.93±0.10 p ₂ <0.05	504.2±5.7 p ₂ <0.05	0.843±0.053 P ₂ <0.05
	Day 21, untreated	1.49±0.05 P ₁ >0.05	10.34±0.36 p ₁ <0.001	4.17±0.08 p ₁ <0.001	379.1±5.4 p ₁ <0.001	0.722±0.042 P ₁ >0.05
	Day 21, MSCs + Corneregel	1.43±0.05 P ₂ >0.05	11.22±0.32 p ₂ <0.05	3.88±0.10 p ₂ <0.05	419.6±5.4 p ₂ <0.001	0.663±0.046 P ₂ <0.05

Notes: p₁ – significance of the difference between the parameter in intact animals and the parameter in animals with untreated corneal burns; p₂ – significance of the difference between the parameter in untreated and treated animals with corneal burns.

Additional indicators of the immune status of the organism and the development of autoimmune processes are the levels of CICs and TNF- α in the blood. Under the conditions of the pathology modeled by us, the concentration of CICs significantly increased throughout the study period in all animals with corneal alkali burns, with the maximum increase on days 7–14 (more than 1.5-fold) and a gradual decrease in their concentration by day 21, but with a 30 % excess over normal. Such dynamics of TNF- α and CIC levels in blood serum indicated an increase in the activity of destructive processes in the cornea.

In the group of animals with corneal alkali burns treated with applications of MSCs modified with monoclonal antibodies against CD73, CD90, CD105, CD34, and CD45, similar dynamics of burn reactive inflammation were observed, but with a significantly accelerated course of the inflammatory process and a decrease in the level of cytotoxic T lymphocytes, which was accompanied by faster normalization of the immunoregulatory index by day 14. A corresponding dynamic was also observed with regard to humoral immune activity: immunoglobulin levels significantly increased during the acute period of corneal burns (days 1–7), gradually decreased, and reached normal values by day 21. A similar dynamic was observed in the concentration of TNF- α , which could indicate an increase in the activity of destructive processes in the cornea during the acute period of the burn and their rapid blockade during the reparative period.

In our observation, a significant increase in the blood level of CICs in burned animals was noted during the acute stage of the process, followed only by a decrease in their concentration without normalization during subsequent stages. Such prolonged circulation of immune complexes in the body may lead to their accumulation in tissues, increased aggregation and adhesion of platelets, which, in turn, causes impairment of blood microcirculation and obliteration of the hemomicrocirculatory bed and tissue damage.

Discussion. Thus, in the acute period of chemical corneal alkali burn, clinical signs developed and were manifested by severe conjunctival hyperemia, corneal stromal edema, and development of dystrophic-necrotic changes in the cornea. Subsequently, the burn process was accompanied by activation of the systemic response of the organism,

intensification of cellular and humoral factors of immune defense, and excessive production of pro-inflammatory cytokines, which significantly affected both the severity of destructive processes in the burn wound and the rate and quality of corneal regeneration. As demonstrated in our previous report [10], during the acute phase of the burn, damaged corneal cells and phagocytes release large amounts of inflammatory mediators and chemotactic factors [4, 13], which leads to a reactive inflammatory process and contributes to epithelial damage and the initial manifestations of corneal neovascularization. At the same time, macrophages and immunocompetent cells infiltrating the burn injury zone stimulate the restoration of the damaged corneal surface and express hematopoietic and chemotactic markers of regeneration, but their hyperactivity leads to endoplasmic reticulum stress, corneal stromal edema, thickening of the corneal stroma, and cessation of epithelial restoration [7].

In addition, it was confirmed that activators of the sanogenetic inflammatory process are potential stimulators of pathological angiogenesis – neovascularization, which is characterized by the formation and proliferation of new vascular capillaries within areas of the cornea that are normally avascular [18]. The development of new, branched, and fragile vessels in corneal tissues is closely associated with persistent and excessive inflammatory processes, active tissue remodeling, and fibrosis, and ultimately neovascularization has a significant negative effect on vision, since immature new blood vessels are “fragile,” bleed, and may lead to persistent inflammation and scar formation, threatening impairment of corneal transparency.

In our study, it was demonstrated that application of MSCs modified with monoclonal antibodies against CD73, CD90, CD105, CD34, and CD45 to an alkali burn injury of the cornea was accompanied by a statistically significant modulation of both cellular and humoral immune responses, which is considered to be associated with differentiation and production of various types of cytokines by stem cells. At the same time, MSCs suppress and accelerate the course of the reactive inflammatory process in the cornea, which reduces the activity of angiogenesis and, ultimately, decreases corneal neovascularization, ensuring preservation of its transparency.

Conclusions

1. In the acute period of chemical corneal alkali burn, clinical signs of a reactive immune-inflammatory stage-specific process develop, with excessive activation of the cellular and humoral responses and dystrophic-necrotic changes in the cornea with formation of PESDs and initial manifestations of neovascularization, which increase over the following three weeks after the burn.

2. Application of pluripotent mesenchymal stem cells modified with monoclonal antibodies against CD73, CD90, CD105, CD34, and CD45 to an alkali burn injury of the cornea leads to a statistically significant modulation of the cellular and humoral components of the immune response, manifested by suppression of the reactive inflammatory process in the cornea, a reduction in angiogenesis and corneal neovascularization activity, which ensures preservation of its transparency.

Prospects for further research: study of optimal doses, routes of administration, and duration of MSC use, assessment of the long-term effects of cell therapy, as well as more detailed study of the mechanisms of interaction between MSCs and corneal cells and the immune system.

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