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ABSTRACT

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MONITORING THE EFFECTIVENESS OF TREATMENT OF CICATRICIAL DEFORMITIES BASED ON THE CHANGES IN TNF-A, MMP-9 AND VEGF LEVELS IN RAT SERUM

The aim of the study: to evaluate the efficacy of therapeutic strategies for scar deformities by determining the levels of TNF-α, MMP-9 and VEGF in the serum of rats during the dynamics of the experiment.

Materials and methods. The experimental part of the study was carried out on 160 white Wistar rats. Four experimental groups were formed: control group – intact animals without surgically induced scar deformities; group I – animals that underwent correction of scar deformities using the gel «Nascar»; group II – animals that underwent correction of scar deformities using the combination of «Nascar»+CO_2-laser; group III – animals that underwent correction of scar deformities using the complex technique of «Nascar»+CO_2-laser+PRP microneedling. To evaluate the efficacy of the treatment, the levels of tumour necrosis factor-alpha (TNF-alpha), matrix metalloproteinase-9 (MMP-9) and vascular endothelial growth factor (VEGF) in rat serum were determined by enzyme-linked immunosorbent assay (ELISA). Blood was collected from the experimental rats by cutting the tip of the tail obliquely in a spiral into a leukocyte melanger with a razor blade.

Results. As a result of the study, it was found that on day 60 of the experiment, the level of TNF-α in the serum of animals in group III («Nascar»+CO_2-laser + PRP microneedling) corresponded to the reference values and was 1.7 times and 1.3 times lower than the same data in group I («Nascar») and group II («Nascar»+CO_2-laser), respectively, $p_1<0.05$, 0.01 and $p_2>0.05$, 0.01, indicating a pronounced anti-inflammatory effect of PRP microneedling. The concentration of VEGF in the blood serum of rats in group III was within physiological limits and was 1.5 and 1.2 times lower than the same values in groups I and II («Nascar»+CO_2-laser), respectively, $p_1<0.01$, $p_2<0.05$. A decrease

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in the activity of matrix metalloproteinases (MMP-9) was found to be 1.7 times and 1.4 times lower than monotherapy with «Nascar» gel (group I) and the combination of «Nascar»+CO_2-laser (group II), respectively, p_1, p_2<0.01;

Conclusions. Thus, the most effective therapeutic approach for correcting scar deformities was the integrated use of a hyaluronic acid-allantoin-onion extract gel, fractional CO_2 laser therapy, and PRP microneedling. This multimodal strategy contributed to the attenuation of systemic inflammation, controlled angiogenesis, balanced neovascularization, and regulated extracellular matrix remodelling, ultimately minimizing the risk of pathological scar formation.

Keywords: scarring deformities, rat serum, TNF-α, MMP-9, VEGF, treatment of scarring changes.

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МОНІТОРИНГ ЕФЕКТИВНОСТІ ЛІКУВАННЯ РУБЦЕВИХ ДЕФОРМАЦІЙ НА ОСНОВІ ДИНАМІКИ РІВНІВ TNF-A, MMP-9 І VEGF У СИРОВАТЦІ КРОВІ ЩУРІВ

Мета дослідження: оцінити ефективність терапевтичних стратегій рубцевих деформацій, шляхом визначення рівнів TNF-α, MMP-9 і VEGF у сироватці крові щурів в динаміці експерименту.

Матеріали і методи. Експериментальна частина дослідження була проведена на 160 білих щурах лінії «Wistar». У рамках експерименту було сформовано чотири піддослідних групи: контрольна група – інтактні тварини без моделювання рубцевих деформацій; I група – тварини, яким корекцію рубцевих деформацій виконували із застосуванням препарату «Nascar»; II група – тварини, у яких корекція рубцевих деформацій здійснювалася з використанням комбінації «Nascar»+CO_2-лазера; III група – тварини, яким проводили корекцію рубцевих деформацій із застосуванням комплексної методики «Nascar»+CO_2-лазера+PRP-мікронідлінгу. Для оцінки ефективності проведеного лікування у сироватці крові щурів визначали концентрацію: фактор некрозу пухлин-α (TNF-α), матриксної металопротеїнази-9 (MMP-9) та фактора росту ендотелію судин (VEGF), методом імуноферментного аналізу (ELISA). Забір крові у піддослідних тварин здійснювали шляхом надрізу бритвою кінчика хвоста навскіс, по спіралі у лейкоцитарний меланжер.

Результати та їх обговорення. У результаті проведеного дослідження встановлено, що на 60 добу експерименту рівень TNF-α у сироватці крові тварин III групи («Nascar»+CO_2-лазер+PRP-мікронідлінг) відповідав референтним значенням та був в 1,7 рази та 1,3 рази нижчим, порівняно з аналогічними даними у I групі («Nascar») та II групі(«Nascar»+CO_2-лазер), відповідно, p_1<0,05, 0,01 та p_2>0,05, 0,01, що вказувало на виражену протизапальну дію PRP-мікронідлінгу. Концентрація VEGF у сироватці крові щурів III групи відповідала фізіологічним межам, та була в 1,5 рази та в 1,2 рази нижче, стосовно аналогічних значень у I та II («Nascar»+CO_2-лазер) групі відповідно, p_1<0,01, p_2<0,05. Встановлено зниження активності матриксних металопротеїназ (MMP-9) у 1,7 рази та 1,4 рази у порівнянні з монотерапією маззю «Nascar» (I група) та

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комбінацією «Nascar» + CO_2-лазер (II група), відповідно, p_1, p_2<0,01.

Висновки. Таким чином, найефективнішим терапевтичним підходом для корекції деформацій рубців було комплексне застосування гелю з гіалуроновою кислотою, алантоїном та екстрактом цибулі, фракційної CO_2-лазерної терапії та мікроголкової терапії з PRP. Ця мультимодальна стратегія сприяла послабленню системного запалення, контрольованому ангіогенезу, збалансованій неоваскуляризації та регульованому ремоделюванню позаклітинного матриксу, що в кінцевому підсумку мінімізувало ризик утворення патологічних рубців.

Ключові слова: рубцеві деформації, сироватка крові щурів, TNF-α, MMP-9, VEGF, лікування рубцевих змін.

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ABBREVIATIONS
TNF-α - tumour necrosis factor-α
MMP-9 - matrix metalloproteinase-9
VEGF - vascular endothelial growth factor
PRP - platelet-rich plasma

INTRODUCTION

Scarring deformities of the soft tissues of the face and maxillofacial area are one of the most pressing problems of reconstructive surgery and dentistry, especially in the context of the growing number of gunshot wounds as a result of the full-scale, shameful invasion of the terrorist country (rf) into Ukraine. The defenders of our country who suffer severe injuries as a result of hostilities often face severe consequences of wound healing, which lead to the formation of pathological scars, soft tissue dysfunction and aesthetic defects that significantly reduce the quality of life and psychological comfort of patients [1, 2].

Scar formation is a complex biological process in which inflammatory mediators, matrix metalloproteinases and angiogenic factors play a key role [3]. Tumour necrosis factor-α (TNF-α) is a major pro-inflammatory cytokine that plays a key role in the regulation of inflammatory processes and the immune response [4, 5]. High levels of TNF-α in the blood are usually a marker for the activation of inflammatory responses associated with tissue damage, fibrosis and impaired healing. As TNF-α actively regulates the phases of healing and tissue remodelling, its level is an important indicator for assessing the effectiveness of scar treatment, particularly in the context of inflammatory and fibrotic processes [6].

Matrix metalloproteinase-9 (MMP-9), also known as hydrolase, is a key enzyme involved in the degradation of extracellular matrix components, including type IV and type V collagen, elastin and other structural proteins [7]. The activity of MMP-9 is critical for the normal process of scar tissue remodelling, as well as for the formation of a new collagen scaffold necessary for the healing of damaged tissue [8]. An imbalance in MMP-9 activity can lead to a disruption of tissue remodelling processes, resulting in the formation of pathological scars such as keloid or hypertrophic scars [9].

Vascular endothelial growth factor (VEGF) is the major regulator of angiogenesis, the process of new vessel formation that is actively stimulated by tissue damage [10]. VEGF plays a key role in regulating the blood supply to scar tissue, ensuring its viability and stimulating healing and regeneration processes [11]. Given these functions, the level of VEGF in the body is an important biomarker for assessing the dynamics of inflammatory and reparative processes, particularly during fibrosis formation [12].

In the context of the treatment of scar deformities in injured soldiers, special attention should be paid to the assessment of these biochemical mechanisms of tissue remodelling, which will allow the use of effective measures to correct pathological scarring in the future.

Given the current challenges associated with the war, the scientific search for optimal methods of treating scarring deformities gains not only medical but also strategic importance [13, 14, 15]. Restoring the health of Ukraine's defenders is an important contribution to our victory, making this topic not only scientifically relevant but also of national importance.

Objective: To evaluate the efficacy of therapeutic strategies for scar deformities by determining the levels of TNF-α, MMP-9 and VEGF in the serum of rats during the dynamics of the experiment.

MATERIALS AND METHODS

The experimental part of the study was conducted in 160 white Wistar rats (males only, 12-14 weeks old, 200-250 g.). A soft tissue defect was modelled on a pre-prepared back area (skin was dissected, a 0.5x1.0 cm skin fragment with muscles was excised using curved-jaw scissors, and the wound was then sutured using «ViCryl» suture material).

Four research groups were formed as part of the experiment: control group – intact animals without surgically induced scar deformities (serving to provide baseline physiological reference values); group I – animals that underwent correction of scar deformities using the gel «Nascar», containing hyaluronic acid, allantoin, and onion extract hereafter referred to as the «gel with active ingredients»; group II – animals that underwent correction of scar deformities using the combination of «Nascar»+CO_2-laser; group III – animals that underwent correction of scar deformities using the complex technique of «Nascar»+CO_2-laser+PRP microneedling. The days in the experiment design were counted from the day of injury and suturing.

«Nascar» gel was applied from the first day of the experiment (subsequent to suturing and washing with Chlorhexidine antiseptic) in a thin layer to the affected area once a day, every other day, throughout the observation period. The utilisation of a neck collar was employed as a preventative measure against autoaggression (biting the suture) and licking off the gel. The gel composed of hyaluronic acid, which promotes fibroblast migration and angiogenesis through enhanced hydration; allantoin, a well-known epithelial stimulant with mild anti-inflammatory properties; and onion extract, which has an inhibitory effect on excessive fibroblast proliferation and collagen synthesis, thereby reducing the risk of hypertrophic or keloid scarring. The polysaccharide base additionally creates a protective film, minimizing microbial contamination and secondary trauma.

The laser therapy was facilitated by means of a fractional CO_2 laser (wavelength 10.6 μm). The energy parameters are as follows: power density 20-25 mJ/cm², fractional microbeam diameter 120 μm, and interval between exposure zones 400 μm. The procedures were performed on the 3rd, 8th, 20th, and 30th days of the experiment.

Platelet-rich plasma (PRP) was prepared using a refrigerated laboratory centrifuge (CM-6 PLASMA) and a vertical laminar cabinet for handling biological samples. The PRP microneedling technique: The PRP was injected intradermally into the affected area following treatment with a CO2 laser, utilising a microneedling device (Dr. Pen M8-W) with 0.5 mm needles. Microneedling was also performed once in each of the control periods on the 3rd, 8th, 20th, and 30th days.

To assess the efficacy of the treatment (on the 7th, 14th, 30th and 60th day of the experiment), the levels of tumour necrosis factor-alpha (TNF- α), matrix metalloproteinase-9 (MMP-9) and vascular endothelial growth factor (VEGF) in rat serum were determined by enzyme-linked immunosorbent assay (ELISA) [16]. TNF-α levels were determined using a commercial TNF-α ELISA kit (Thermo Fisher Scientific, USA), the reference values for TNF-α in healthy rats are: 5–20 pg/ml; MMP-9 using a highly sensitive commercial MMP-9 rat ELISA kit (Abcam, UK), the reference values for MMP-9 in healthy rats are: 5–15 ng/ml; VEGF using a commercial VEGF rat ELISA kit (R&D Systems, USA), the reference values for VEGF in healthy rats are: 20–40 pg/ml [17, 18, 19]. Blood was collected from experimental rats by obliquely cutting the tip of the tail and spiralling it into a leukocyte melanger with a razor [20].

The experimental part of the study was conducted in accordance with the principles of the Helsinki Declaration for the humane treatment of animals, the principles of the European Community Directive (86/609/EC), the principles of the European Convention for the Protection of Vertebrate Animals (European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS 123), Strasbourg, 1986), and the basic provisions of the «General Ethical Principles for Animal Experimentation», (European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS 123) Strasbourg, 1986), and the basic provisions of the «General Ethical Principles for Animal Experimentation» approved by the First National Congress on Bioethics (Kyiv, 2001) and the basic tenets of the Law of Ukraine № 3447-IV «On the Protection of Animals from Cruelty». 3447-IV «On Protection of Animals from Cruelty». The procedures were approved by the Bioethics Committee of the Bukovinian State Medical University. The work was performed without the involvement of grants and government funding.

Statistical data processing was carried out using licensed Microsoft Excel 2016 software. The data were structured by contingent characteristics, which allowed the calculation of relative and mean values, the estimation of their errors and the use of the t-test. The

critical significance level was set at 0.05 to test statistical hypotheses.

RESULTS AND DISCUSSION

On the basis of the data obtained, it was established (Fig. 1) that at the early stage of the experiment (day 7) in all animals of the experimental groups there was a sharp increase in the level of TNF-α in the blood serum compared with the data of the control group, $p<0.01$, indicating a pronounced inflammatory reaction at the site of injury. The maximum levels of TNF-α (148.56±5.94 pg/ml) were observed in the serum of group I animals (treated by gel with active ingredients) and were 17.2 times higher than in the control group, $p<0.01$. The combined application of gel with active ingredients and CO_2-laser therapy (group II) contributed to a moderate decrease in the level of proinflammatory cytokines in the blood to 115.26±5.73 pg/ml, which was 14.3 times higher than in the control group, $p<0.01$, but 1.3 times lower than in group I, $p_1<0.01$. The lowest levels of TNF-α in the blood serum of the experimental animals (97.88±5.26 pg/ml) were recorded in group III, where PRP microneedling was additionally included in the treatment. However, this value was 11.9 times higher than the same data in the control group, $p<0.01$, but at the same time 1.5 and 1.2 times lower than the values in groups I and II, respectively, $p_1<0.01$, $p_2<0.05$.

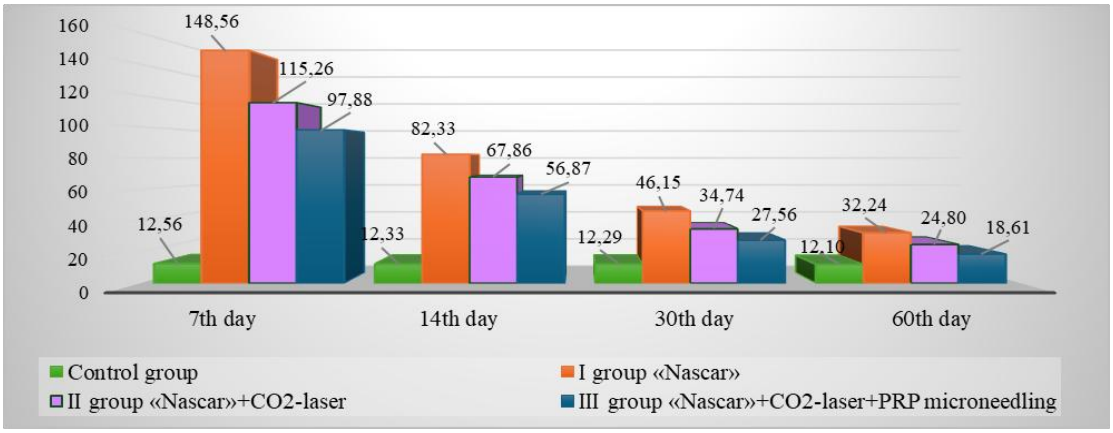

Figure 1 – TNF-α values (pg/ml) in rat serum in the dynamics of the experiment

Although PRP microneedling is itself a microtraumatic procedure and may induce a transient local elevation of TNF-α, our blood samples were collected at defined control points (days 7, 14, 30, and 60) rather than within the first hour's post-intervention. By these later intervals, the anti-inflammatory cytokine cascade triggered by PRP-derived growth factors (PDGF, TGF-β, VEGF, EGF) had already attenuated the acute inflammatory phase. Consequently, group III demonstrated significantly lower systemic TNF-α levels, reflecting a more regulated inflammatory resolution rather than a lack of tissue response.

On day 14 of the experiment, a significant decrease in blood TNF-α levels was observed in all experimental groups, but the levels remained significantly higher than the control values, $p<0.01$. In group I animals, the level of TNF-α in the blood serum decreased to 82.33±5.42 pg/ml, which was 9.5 times higher than in the control group, $p<0.01$. In group II («gel with active ingredients»+CO_2-laser) the level of this cytokine was 67.86±4.75 pg/ml, which was 7.8 times higher than in the control group, $p<0.01$, and 1.2 times lower than in group I, $p_1<0.05$. In the blood of animals of experimental group III («gel with active ingredients»+CO_2-laser+PRP microneedling), the level of TNF-α decreased to 56.87±4.30 pg/ml, which was 6.5 times higher than in the control group, but 1.4 and 1.2 times lower than in groups I and II, respectively, $p_1<0.01$, $p_2<0.05$.

On day 30 of the experiment, the level of TNF-α in the blood serum of the experimental animals continued to decrease, indicating a gradual transition from the inflammatory process to the regenerative phase. In the blood of group I («gel with active ingredients») animals, the level of this cytokine was 46.15±2.60 pg/ml, which was 3.8 times higher than in the control group, $p<0.01$. In the experimental animals of group II («gel with active ingredients»+CO_2-laser), the level of TNF-alpha decreased to 34.74±2.25 pg/ml, which was 2.9 times higher than in the control group, $p<0.01$, and 1.3 times lower than in group I, $p_1<0.01$. The lowest level of TNF-α was observed in the blood of animals of group III («gel with active ingredients»+CO_2-laser+PRP microneedling) – 27.56±2.48 pg/ml, which was 2.3 times higher than in the control group, $p<0.01$, but at the same time 1.7 and 1.3 times lower than in groups I and II, respectively, $p_1<0.01$, $p_2<0.05$.

On day 60 of the experiment, TNF-α levels continued to decrease in all groups, but remained slightly higher than in the control group. In the animals

of group I («gel with active ingredients»), the average value of this indicator was 32.24±2.41 pg/ml, which was 2.7 times higher than in the control group, p<0.01. The application of the «gel with active ingredients»+CO_2-laser complex made it possible to reduce the level of TNF-α in blood serum to 24.80±2.35 pg/ml, although it exceeded the value in the control group by 2.0 times, p<0.01, but was 1.3 times lower than in group I, p_1<0.01. It is noteworthy that in animals of group III («gel with active ingredients»+CO_2-laser+PRP microneedling) the concentration of TNF-α was 18.61±2.06 pg/ml and exceeded the data in the control group by 1.5 times, p<0.01, but at the same time it was 1, 7 and 1.3 times lower compared to similar values in groups I and II, respectively, p_1<0.01, p_2<0.05, which indicates complete extinction of the inflammatory process and acceleration of regeneration processes.

As a result of the study, it was found (Table 1) that on day 7 after modelling the defect in all experimental groups (I, II, III) there was a significant increase in the level of MMP-9 compared to intact animals, p<0.01. The highest level of this enzyme was found in group I («gel with active ingredients»), where the concentration of MMP-9 was 3.0 times (p<0.01) higher than in the control group. In the blood serum of animals in group II («gel with active ingredients»+CO_2-laser therapy), the level of MMP-9 was 1.2 times lower than in group I, p_1<0.05, but still exceeded the value in the control group by 2.64 times, p<0.01, indicating the anti-inflammatory effect of laser exposure. The lowest levels of MMP-9 during this period were observed in the serum of animals in group III («gel with active ingredients»+CO_2-laser+PRP microneedling), while the level of the enzyme remained 2.3 times higher than in the control group, p<0.01. At the same time, the level of MMP-9 in the blood serum of group III animals was 1.3 and 1.2 times lower, respectively, compared to similar values in groups I and II, p_1<0.01, p_2<0.05.

Table 1 – The value of MMP-9 (ng/ml) in the blood serum of rats in the dynamics of the experiment

Research groups	Observation periods			
	7th day	14th day	30th day	60th day
Control group, intact animals, (n=40)	10,37 ± 0,84	12,15 ± 0,88	11,28 ± 0,79	9,65 ± 0,76
I group «Nascar», (n=40)	31,47 ± 1,52 •	26,83 ± 1,49 •	23,69 ± 1,21 •	19,05 ± 1,03 •
II group «Nascar»+CO_2 laser, (n=40)	27,42 ± 1,31 •,**	22,07 ± 1,14 •,**	20,54 ± 1,01 •,**	16,25 ± 0,93 •,**
III group «Nascar»+CO_2 laser+PRP microneedling, (n=40)	23,87 ± 1,14 •,*,††	18,45 ± 1,22 •,*,††	17,28 ± 1,02 •,*,††	11,51 ± 0,75 ••,*,†

Notes:

•p<0,01; ••p<0,05 – significant difference in values relative to the data in the control group;

**p_1<0,01; **p_1<0,05 – significant difference in values relative to the data in group I;*

†p_2<0,01, ††p_2<0,05 – significant difference in values relative to the data in group II

On the 14th day of the experiment, there was a tendency for the level of MMP-9 in the blood serum to decrease in all experimental groups, indicating a gradual disappearance of the inflammatory response. Thus, in the serum of animals of group I, in which only the gel with active ingredients was used for the treatment of scar deformities, a minimal decrease of this indicator was recorded, which was 2.2 times higher (p<0.01) compared to the values of the control group. In the animals of group II («gel with active ingredients»+CO_2-laser therapy), the level of MMP-9 in blood serum during this observation period was 1.8 times higher than in the control group, p<0.01, but at the same time it was 1.2 times lower than in group I, p_1<0.05. The maximum decrease in the level of MMP-9 in the blood was observed in group III, although its concentration remained 1.5 times higher than in the control group, p<0.01. It is noteworthy that the values of this parameter in group III were 1.5 and 1.2 times lower than in groups I and II, respectively, p_1<0.01, p_2<0.05.

On the 30th day of observation, the level of MMP-9 in the blood serum of the experimental animals continued to decrease in all groups. Thus, in group I animals treated by gel with active ingredients alone, this indicator still exceeded the value in the control group by 2.1 times, p<0.01, which may indicate the preservation of the activity of the scarring process. The use of CO_2 laser in combination by gel with active ingredients in group II contributed to a better dynamics of reduction of MMP-9 level, although it was 1.8 times higher, p<0.01, compared to the values in the control group, and 1.2 times lower compared to similar values in group I, p_1<0.05. The

lowest level of this enzyme was recorded in group III («gel with active ingredients»+CO_2-laser+PRP microneedling), where the MMP-9 index exceeded the value in the control group only by 1.5 times, $p<0.01$, but was statistically significantly lower – by 1, 4 and 1.2 times compared to the same data in groups I and II, respectively, $p_1<0.01$, $p_2<0.05$, which again indicates the optimal rate of remodelling of damaged tissue in this group.

On day 60 of the experiment, MMP-9 levels in the experimental groups approached control levels, indicating the completion of the major regeneration processes. At the same time, the level of MMP-9 in the blood serum of group I animals, in which scar deformities were treated by gel with active ingredients, remained 2.0 times higher than in the control group, $p<0.01$, which may indicate the risk of dense scar formation. The use of CO_2-laser therapy in combination with gel reduced the level of the enzyme, but it was still 1.7 times higher than in the control group, $p<0.01$, but at the same time it was 1.2 times lower than in group I, $p_1<0.05$. In group III («gel with active ingredients»+CO_2-laser+PRP microneedling), the level of MMP-9 in blood serum was still 1.3 times higher than in the control group, $p<0.05$, but it was in line with reference values, indicating the completion of reparative processes and minimisation of the risk of pathological scarring. It is noteworthy that the values of this indicator in group III were 1.7 and 1.4 times lower than in groups I and II, respectively, p_1, $p_2<0.01$. This indicates that the complex promotes a more controlled remodelling of scar tissue and reduces the risk of its excessive restructuring.

Analysis of the results obtained allowed us to establish (Fig. 2) that on the 7th day of the experiment in all experimental groups, the level of vascular endothelial growth factor (VEGF) in the blood serum was significantly higher than in the control group, $p<0.01$, which could indicate the activation of angiogenesis in response to tissue damage. Thus, the maximum increase of this indicator in blood serum (58.83±2.16 pg/ml) was objectified in rats of group I, where only gel was used to correct scar deformities, and its concentration was 2.6 times higher than in the control group, $p<0.01$. In the animals of the second experimental group («gel with active ingredients»+CO_2-laser), this indicator was slightly reduced (51.64±2.00 pg/ml), although it was 2.3 times higher than in the control group, $p<0.01$, but 1.2 times lower than in the first group ($p_1<0.01$). The lowest level of VEGF in the blood serum of the experimental animals was recorded in group III (45.45±2.22 pg/ml), where the correction of scar deformities was performed by the combination of «gel with active ingredients»+CO_2-laser+PRP microneedling, and was 2.0 times ($p<0.01$) higher than in the control group. At the same time, the value of this parameter in group III was 1.3 and 1.1 times lower than in groups I and II, respectively, $p_1<0.01$, $p_2<0.05$, which may be due to the intense activation of endothelial cells in response to tissue damage and the need to form new microvessels to ensure reparative processes.

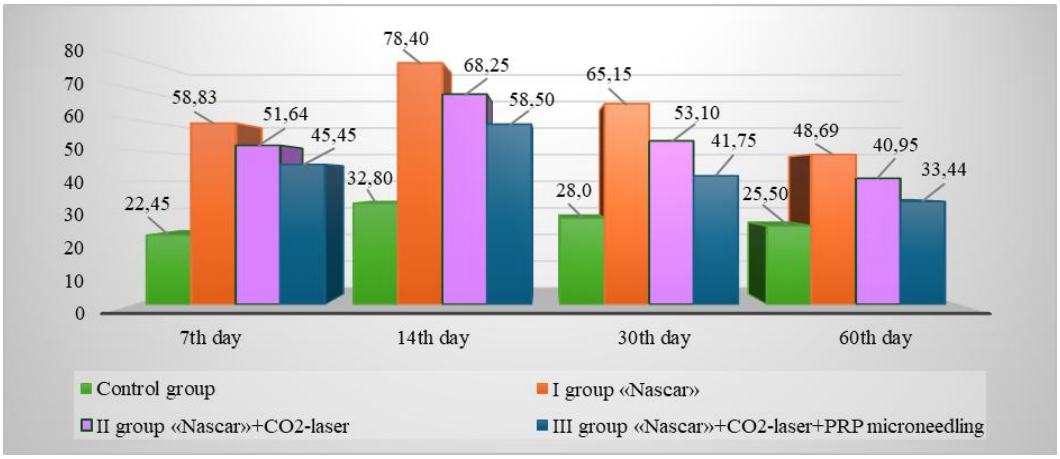

Figure 2 – VEGF values (pg/ml) in rat serum in the dynamics of the experiment

On the 14th day of the experiment, a further increase in blood VEGF levels was observed in all experimental groups, indicating the peak phase of angiogenesis. The highest values of this parameter were observed in rats of group I (78.40±2.32 pg/ml), which were 2.4 times, $p<0.01$, higher compared to similar data in the control group. In the animals of group II («gel with active ingredients»+CO_2-laser), the level of VEGF in the blood during the given observation period was 68.25±2.22 pg/ml, which was 2.1 times higher than the data in the control group, $p<0.01$, but at the same time it was 1.2 times lower, $p_1<0.05$, relative to similar values in group I. Inclusion of PRP microneedling promoted even more pronounced decrease of VEGF level in group III

(«gel with active ingredients»+CO_2-laser+PRP microneedling), relative to the values in experimental groups I and II (1.3 and 1.2 times, respectively), $p_1<0.01$, $p_2<0.05$, but still exceeded the similar data (1.8 times) in control group, $p<0.01$. The growth of VEGF in the blood at this stage of the study is typical of the process of remodelling the vascular network, which helps to improve the vascularisation of the damaged area and supply it with the necessary trophic factors.

At day 30, a gradual decrease in VEGF levels was observed in all experimental groups, indicating the completion of the proliferative phase of angiogenesis and the gradual differentiation of the newly formed vessels, ensuring the transition from active growth to stabilisation of the microcirculation. Thus, in animals of group I (gel with active ingredients), the level of VEGF in the blood serum remained the highest among the experimental groups and was 65.15±2.08 pg/ml, which was 2.3 times higher than in the control group, $p<0.01$. In the blood serum of rats of group II («gel with active ingredients»+CO_2-laser), the average value of this indicator was 53.10±2.57 pg/ml, which was 1.9 times higher than in the control group, $p<0.01$, but 1.2 times lower than in the experimental animals of group I, $p_1<0.05$. The lowest level of VEGF in the blood of the experimental groups was found in animals of group III («gel with active ingredients»+CO_2-laser +PRP microneedling) - 41.75±2.78 pg/ml, which was 1.5 times, $p<0.01$, higher than in the control group. At the same time, the concentration of VEGF in the blood serum of group III animals was found to be 1.6 and 1.3 times lower than in group I and II animals, respectively, $p_1<0.01$, $p_2<0.05$.

On day 60 of the experiment, the levels of VEGF in the blood serum of the experimental animals continued to decrease in all groups, approaching the levels of the control group, which may indicate the completion of the angiogenic response and the stabilisation of scar tissue vascularisation. Thus, in group I animals treated with «Nascar» gel alone, the VEGF level in the blood serum was 48.69±2.80 pg/ml, which was 1.9 times higher than in the control group, $p<0.01$. In animals of experimental group II, where scar deformities were treated with the combined use of «Nascar» gel and CO_2-laser therapy, the level of VEGF decreased to 40.95±2.04 pg/ml, which was 1.2 times lower than in animals of group I, $p_1<0.05$, but still exceeded the control value by 1.6 times, $p<0.05$. The lowest level of VEGF in the blood of the experimental groups was recorded in experimental group III (33.44±2.52 pg/ml), which received complex treatment with the use of «Nascar»+CO_2-laser+PRP microneedling. During this period of the study, the level of VEGF in the blood was within the reference limits, although it remained 1.3 times higher than in the control group, $p<0.05$. It is also worth noting that the concentration of this indicator in the blood serum of group III animals was 1.5 and 1.2 times lower than in groups I and II respectively, $p_1<0.01$, $p_2<0.05$, indicating a more controlled angiogenic response when using «gel with active ingredients»+CO_2-laser+PRP microneedling.

It is important to emphasize that the lowest systemic levels of TNF-α and MMP-9 observed in the PRP microneedling group do not indicate an absence of reparative activity. Rather, they reflect a controlled downregulation of excessive inflammation and matrix degradation, accompanied by balanced angiogenesis and extracellular matrix remodelling. This finding is consistent with histological observations [21], where the PRP group demonstrated more organized collagen deposition and reduced fibrotic density compared with other groups. Thus, the combination of PRP with laser therapy and topical gel should be regarded not as suppressive, but as modulatory, ensuring a more favorable scar remodelling trajectory.

CONCLUSIONS

Thus, the most effective therapeutic approach for correcting scar deformities was the integrated use of a hyaluronic acid-allantoin-onion extract gel, fractional CO_2 laser therapy, and PRP microneedling. This multimodal strategy contributed to the attenuation of systemic inflammation, controlled angiogenesis, balanced neovascularization, and regulated extracellular matrix remodelling, ultimately minimizing the risk of pathological scar formation.

PROSPECTS FOR FUTURE RESEARCH

In future studies, particular attention will be paid to the temporal kinetics of PRP-induced cytokine modulation, including early post-procedural surges in pro-inflammatory mediators, as well as detailed histological correlations. This will further clarify the paradox of transient microtrauma yielding long-term regenerative benefits.

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A –Work concept and design,
B –Data collection and analysis,
C –Responsibility for statistical analysis,
D –Writing the article,
E –Critical review,
F –Final approval of the article.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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