

UDC 616-001.4-002.2-092.9:615.831

DOI <https://doi.org/10.32782/2226-2008-2025-6-12>

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REGULATORY EFFECTS OF 660 NM PHOTOBIO-MODULATION IN WOUND HEALING: TOWARD PARAMETER OPTIMIZATION

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Introduction. Photobiomodulation (PBM) therapy is a promising approach to wound healing because of its potential to accelerate tissue repair. However, the mechanisms of light interaction with tissue are not fully understood.

The work **aims** to study the effect of PBM therapy on the expression of biomolecules regulating the repair processes of chronic wounds at the remodeling stage, using ROS, IL-1 β , IL-6, IL-4, and TNF- α as examples.

Materials and methods. The experiment involved 18 rats randomized into intact, control, and experimental groups. The animals of the control and experimental groups have been modeled with a chronic wound. The wound defects of the rats in the experimental group were exposed to PBM therapy (wavelength 660 nm, power 50 mW, energy density 5 J/cm²). Animals were euthanized on day 21 of the experiment. ROS, IL-1 β , IL-6, IL-4, and TNF- α levels were determined using an enzyme-linked immunosorbent assay in the blood serum.

Results. Our study showed that PBM therapy increased levels of ROS, IL-1 β , IL-6, IL-4, and TNF- α during the remodeling stage. Thus, in rats on the 21st day of the experiment, serum levels of ROS were increased 1.36-fold, IL-4 – 1.73-fold, IL-1 β – 1.19-fold, TNF- α – 1.17-fold, and IL-6 – 1.53-fold compared to the same indices of animals with wounds without treatment.

Conclusions. PBM can increase the expression of biomolecules, regulating the processes of chronic wound repair during the remodeling stage.

Keywords: photobiomodulation, reparative process, wound healing, remodeling, cytokines.

УДК 616-001.4-002.2-092.9:615.831

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РЕГУЛЯТОРНІ ЕФЕКТИ ФОТОБІОМОДУЛЯЦІЇ З ДОВЖИНОЮ ХВИЛІ 660 НМ У ЗАГОЄННІ РАН: НА ШЛЯХУ ДО ОПТИМІЗАЦІЇ ПАРАМЕТРІВ

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Досліджено вплив фотобіомодуляційної (ФБМ) терапії на експресію біомолекул, що регулюють репараційні процеси хронічних ран, на стадії ремоделювання. В експерименті використано 18 шурів. Вплив ФБМ терапії вивчали на моделі хронічної рани. Застосовувалися такі параметри ФБМ терапії, як: довжина хвилі 660 нм, потужність 50 мВт, щільність енергії 5 Дж/см². ФБМ терапія призвела до підвищення рівнів досліджуваних параметрів на 21-у добу експерименту, що демонструє дисбаланс експресії про- і протизапальних цитокінів на стадії ремоделювання загоєння ран.

Ключові слова: фотобіомодуляція, репаративний процес, загоєння ран, ремоделювання, цитокіни.

Introduction

Chronic wounds, despite advances in their treatment, negatively affect patients' quality of life and lead to socio-economic difficulties. Usually, these wounds commonly occur as a result of population aging, comorbidities, immune and endocrine system disorders, stress, etc. [1; 2].

The search for methods to influence wound healing remains highly relevant. Potential solutions to improve chronic wound care include negative pressure wound therapy, systemic hyperbaric oxygen therapy, nanotherapy, and stem cell therapy [3]. Physical methods have also found their way into the treatment of injuries [4; 5]. Photobiomodulation (PBM) therapy is a promising approach to wound healing because of its potential to accelerate tissue repair. PBM therapy has been applied in the treatment of many diseases [6]. However, the mechanisms of light interaction with tissue are not fully understood. It is also necessary to

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determine the role and optimal settings of PBM parameters.

The **purpose** of the work is to study the effect of PBM therapy on the expression of biomolecules regulating the repair processes of chronic wounds at the remodeling stage, using reactive oxygen species (ROS), interleukin-1 β (IL-1 β), interleukin-6 (IL-6), interleukin-4 (IL-4), and tumor necrosis factor-alpha (TNF- α) as examples.

Materials and Methods

The study was performed on 18 rats weighing approximately 220 g, randomized into three groups (intact (Int), control (Con), and experimental (Exp)) with 6 animals in each group. Animals in the intact group received no interventions. The effect of PBM therapy was studied on the chronic wound model. For this purpose, the animals of the control and experimental groups were given a 2-cm-diameter wound. The surgical intervention was performed under aseptic conditions on a pre-depilated area of the rat's back. A perpendicular loop-shaped skin-fascial suture was applied along the edges of the wound. On the surface of the wound bed, the superficial fascia was incised with perpendicular incisions to form 5 \times 5 mm areas, which were sutured with U-shaped sutures. Anesthesia was used via intramuscular injection of zoletil solution (tiletamine hydrochloride and zolazepam hydrochloride) (Virbac, France) at a rate of 10 mg/kg body weight. The wound defects of the rats' experimental group were exposed to PBM therapy. The laser device Lika therapist M (Ukraine) was used. The following parameters were applied: continuous mode, wavelength 660 nm, power 50 mW, energy density 5 J/cm², exposure time 314 s. PBM therapy was applied perpendicularly and covered the entire wound area for 5 days. During therapy, the laser tip was held at a distance of 3.53 cm from the animal's wound defect. The treatment was started on the next day after wound induction. Animals were euthanized on day 21 of the experiment. Blood sampling was performed by open cardiac puncture. ROS, IL-1 β , IL-6, IL-4, and TNF- α levels were determined using enzyme immunoassay in the blood serum. Concentrations of IL-1 β , IL-6, IL-4, and TNF- α were measured using Vector-Best reagent kits (Ukraine). ROS levels were determined using the Elabscience kit (USA). To assess the dynamics of wound size, digital macrophotographs of the wound surface were taken in animals from the control and experimental groups. The area of the wound surface was estimated from photographic images using ImageJ software (NIH, USA). The relative wound area (S) was calculated according to the formula:

$$S = St / So \times 100 \%,$$

where So is the wound area after it has been inflicted; St is the surface area of the wound at a given healing period.

During the experiment, the animals were kept in the Kharkiv National Medical University vivarium with a controlled temperature regime, on a standard diet with free access to water and food, and a 12-hour day-night cycle. The experimental study was conducted by the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and the Law of Ukraine "On the Procedure for Conducting Experiments

and Experiments on Animals by Scientific Institutions" (No. 249 of 03/01/2012). The study was also approved by the Ethics and Bioethics Committee of Kharkiv National Medical University, Ukraine (Protocol No. 17, dated June 3, 2024).

Statistical analyses were performed using Statistica 12.0 software (StatSoft, USA). After checking the normality and homoscedasticity of the data, the effect of PBM on the selected parameters was analyzed using one-way ANOVA. Normality within each group was assessed using the Shapiro–Wilk test ($p > 0.05$). The assumption of homogeneity of variances was verified using Levene's test ($p > 0.05$). Therefore, despite the small sample size, it met the requirements for applying this statistical method. Tukey's HSD test was used to assess significant differences between groups. The obtained results were expressed as mean values \pm standard error (SE). The significance level for all tests was set at $p < 0.05$. Histogram plotting was performed in GraphPad Prism 9 (GraphPad Software, USA).

Research results and their discussion

Fig. 1 shows the concentrations of bioactive molecules in the animals' blood serum on the 21st day of the experiment.

Our study showed that under the influence of PBM therapy, serum ROS levels were 1.36-fold higher ($p < 0.01$) in rats on day 21 of the experiment compared to animals with wounds without treatment (Fig. 1A). It was also found that the ROS level in animals of the experimental group was elevated 1.54 times ($p < 0.001$) compared to intact animals.

Animals exposed to PBM therapy showed a 1.73-fold ($p < 0.001$) increase in serum IL-4 levels compared to the control group (Fig. 1B). At the same time, while the concentration of IL-4 in the experimental group increased by 1.78-fold ($p < 0.001$) compared to intact animals, no differences in the indices of this cytokine in the control and intact groups were found.

In our study, 21 days after surgery, PBM therapy resulted in a 1.19-fold increase in serum levels of pro-inflammatory IL-1 β ($p < 0.01$) compared to animals without PBM therapy (Fig. 1C). It was also found that the IL-1 β level in animals of the experimental group was elevated 1.63 times ($p < 0.001$) compared to intact animals. At the same time, the concentration of this cytokine was 1.37-fold higher in the control group compared to intact rats ($p < 0.001$).

According to the data obtained, there was an increase in TNF- α concentration on the 21st day after wound modeling in the group with PBM by 1.17 times ($p < 0.01$) compared to animals without PBM therapy (Fig. 1D). It also showed an increase in TNF- α levels in animals of the experimental group by 1.67-fold ($p < 0.001$) and control group by 1.42-fold ($p < 0.001$) compared to intact animals.

The application of PBM therapy resulted in a 1.53-fold ($p < 0.001$) increase in serum IL-6 levels in the experimental group compared to the control group and 2.57-fold ($p < 0.001$) compared to intact animals (Fig. 1E). The concentration of this cytokine was 1.68-fold higher in the control group compared to the intact group ($p < 0.001$).

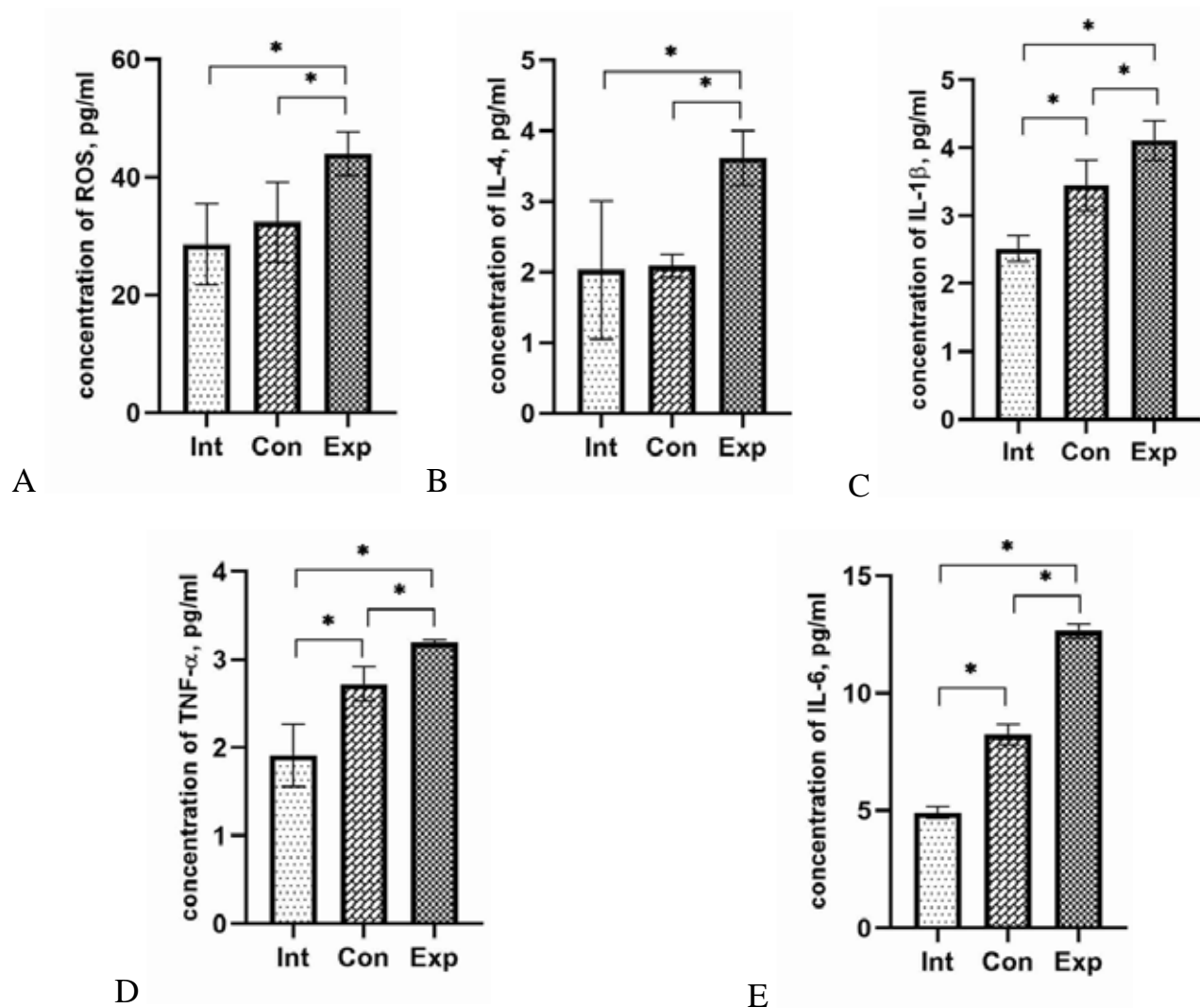


Fig. 1. Changing the levels of the studied biomarkers in the blood serum of animals: (A) ROS, (B) IL-4, (C) IL-1 β , (D) TNF- α , (E) IL-6 (*p < 0.05). Int – intact animals, Con – control group, Exp – experimental group. The error bars represent the standard error of the arithmetic mean for each indicator (n = 6)

The results of measuring the relative area of the wound surface in the control and experimental groups of animals are presented in Table 1.

The results of our study demonstrated a reduction in wound healing time when using PBM therapy in the early stages of healing. On the third day, the wound area in the experimental group was significantly smaller than in the control group. However, starting from day 10 of the experiment, the relative area of the wound surface in the experimental group was greater than that of the control animals, although this difference was not statistically significant.

Wound healing progresses through several overlapping stages: inflammation, proliferation, and remodeling. Since the impact of PBM therapy in the early healing phase on the subsequent phases has not been sufficiently studied, we focused on investigating the remodeling phase. The remodeling phase of wound healing begins two to three weeks after the initial injury. During this phase, scar tissue develops, blood vessel density decreases, and collagen is organized and modified.

A wound model characterized by local hypoxia and impaired microcirculation was used to study reparation processes. Taking these criteria into account, as well as the prolonged inflammatory phase and impaired healing, the wound model can be regarded as chronic. Hypoxia is known to impair angiogenesis and reduce cell signaling, which can delay wound healing [7]. In our work, ROS levels increased in response to PBM therapy using the applied parameters. ROS are known to play a pleiotropic role in wound healing. Excessive levels of ROS can induce oxidative stress, contributing to the pathogenesis of chronic wounds. Moderate ROS levels promote the modulation of multiple cellular signaling pathways to promote fibroblast and keratinocyte proliferation, migration and differentiation, and angiogenesis, thereby promoting collagen remodeling and extracellular matrix formation [8].

IL-4 and IL-13 are known to promote effective angiogenesis and collagen synthesis by fibroblasts [9]. In our work, IL-4 levels were increased in the experimental group using PBM therapy. Previously, based on histological analysis, an increase in collagen production as well as a

Table 1

Changes in the relative area of the wound surface

Relative area of the wound surface in the groups, %	Days								
	3	5	7	10	12	14	16	18	21
Con	93.50 ± 1.64	82.90 ± 3.04	78.12 ± 3.11	65.60 ± 2.86	57.02 ± 2.46	47.83 ± 3.91	35.98 ± 5.00	23.15 ± 3.26	10.32 ± 2.30
Exp	84.58 ± 1.50*	75.63 ± 1.38	71.40 ± 1.00	66.30 ± 1.41	62.33 ± 1.36	56.78 ± 1.08	45.30 ± 1.80	29.62 ± 2.00	14.10 ± 1.32

* – compared to the group Con.

decrease in the number of newly formed vessels in animals whose wounds were exposed to laser irradiation was shown at an earlier time compared to the control group [10].

Chronic wounds are prone to prolonged inflammation. A dynamic balance between pro- and anti-inflammatory mediators is necessary to prevent excessive inflammation and tissue damage. The levels of pro-inflammatory cytokine IL-1β were increased in our study after exposure to PBM therapy. IL-1β increases oxidative stress and induces inflammation, cell migration and proliferation, and endothelial dysfunction [11]. It has been previously shown that PBM therapy using different parameters (660 nm, 1 J/cm², 10 mW power) during the remodeling phase did not alter ROS and IL-1β levels [12].

Our work shows that TNF-α levels increased after exposure to PBM therapy. TNF-α is a pleiotropic cytokine. It can inhibit the wound-healing process in high concentrations by degrading extracellular matrix formation during the remodeling phase of wound healing [13]. The literature reports conflicting findings on the effect of PBM therapy on TNF-α concentrations. Thus, using red light (625 nm, 4 J/cm²) for chronic venous ulcers did not reduce local TNF-α levels [14]. At the same time, there is data in the literature on reducing TNF-α levels after exposure to PBM therapy in wound healing [15].

The study of pleiotropic IL-6, a key regulator of inflammation and repair, has been important for wound healing [16]. IL-6 can regulate the differentiation of fibroblasts into myofibroblasts [17]. *In our study, the use of PBM therapy increased IL-6 levels in the serum of experimental animals.* The literature shows that PBM can alter IL-6 release dose-dependently: high doses suppress IL-6 production, while low doses enhance IL-6 release [18]. Thus, considering the dose-effect relationship of laser radiation, determining the optimal parameters is a crucial

task in the application of PBM therapy. New dosages should be tested to achieve better results.

The study of biomarker ratios, such as IL-1β/IL-1RA and CXCL8/CXCL10, at different stages of wound healing [19] represents a promising direction that may provide additional diagnostic and prognostic value in the assessment of impaired healing in the future.

An important prognostic factor for wound healing is the assessment of the wound surface area dynamics. In our study, on day 21 of the experiment, there were no statistically significant differences in wound reduction in rats in the experimental group compared to the control group. Similar results were obtained by C. de Loura Santana et al., where, beginning with day 8, by day 22 of the experiment, there were no statistically significant differences in injury area between the PBM groups and the control group [20]. The variety of parameters and methodologies complicates the comparison of research results and indicates the need for standardization in the use of PBM therapy.

Conclusions

The use of PBM therapy resulted in increased levels of ROS, IL-1β, IL-6, IL-4, and TNF-α during the remodeling stage. This indicates an imbalance in the expression of pro- and anti-inflammatory cytokines.

There is a need to identify optimal parameters of PBM therapy for better wound healing. This will be the basis for further refinement and practical application of the findings in the development of a therapeutic approach.

Conflicts of Interest. The authors declare that there is no conflict of interest regarding the publication of this article.

Funding. The Ministry of Health of Ukraine funded this research from the state budget (state registration No 0124U000579, 2024–2026).

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Надійшла до редакції 21.04.2025

Прийнята до друку 02.02.2026

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