

Anti-inflammatory and cell proliferative effect of the 1270 nm laser irradiation on the BALB/c Nude mouse model involves activation of the cell antioxidant system

DINARA DOLGOVA¹, TATIANA ABAKUMOVA¹, TATIANA GENING¹, LUDMILA POLUDNYAKOVA¹, IGOR ZOLOTOVSKII², DMITRII STOLIAROV², ANDREI FOTIADI^{2,3,4}, ANNA KHOKHLOVA², EDIK RAFAILOV^{3,5,*}, SERGEI SOKOLOVSKI^{3,6,*}

¹ Department of Physiology and Pathophysiology, Faculty of Medicine, Ulyanovsk State University, 42 Leo Tolstoy Street, Ulyanovsk 432017, Russia

² S.P. Kapitsa Technological Research Institute, Ulyanovsk State University, 42 Leo Tolstoy Street, Ulyanovsk 432017, Russia

³ Aston Institute of Photonic Technologies, Aston University, Aston Triangle, Birmingham B4 7ET, U.K.

⁴ Electromagnetism and Telecommunication Department, University of Mons, 31 Boulevard Dolez, 7000 Mons, Belgium

⁵ Interdisciplinary Center of Critical Technologies in Medicine, Saratov State University, 83 Astrakhanskaya Street, Saratov 410012, Russia

⁶ Laboratory of Optics and Dynamics of Biological Systems, Novosibirsk State University, 2 Pirogova Street, Novosibirsk 630090, Russia

* e.rafaïlov@aston.ac.uk

* s.sokolovsky@aston.ac.uk

Abstract

Recently, a lot of interdisciplinary community researchers have focused their efforts on study of the low-level light irradiation effects (photobiomodulation (PBM)) as a promising therapeutic technology. Among the priorities, a search of new wavelength ranges of laser radiation to enhance the laser prospects in treatment of autoimmune and cancer diseases commonly accompanied by disorders in the antioxidant system of a body. Therefore, PBM effects within the wavelength range of 1265-1270 nm on a model organism are of particular interest for preclinical studies, as this range corresponds to the maximum oxygen absorption band. Balb/c Nude mice are selected as an object for a comprehensive assessment of changes registered after a single exposure of mouse skin to a continuous laser at the 1270 nm wavelength and energy densities of 0.12 and 1.2 J/cm². A histological analysis has been performed and the miRNA expression, formation of lipid peroxidation products, level of antioxidant enzymes (catalase and glutathione), as well as level of cytokines (IL-6 and TNF- α) have been determined. The morphometric analysis of skin after irradiation at the energy density of 1.2 J/cm² shows an increase in the number of basal layer epithelial cells; the growth layer cells have elongated nuclei and in some places demonstrate overlapping. MiRNA expression assay reveals changes in the expression of miR-31 and miR-200c regulating post-transcriptional activity of genes controlling the division of keratinocytes and differentiation of other cells. Biochemical blood test has demonstrated a significant increase in the reduced glutathione level. Also, the changes in the levels of membrane lipid oxidation products are observed. A decrease in the level of anti-inflammatory cytokine IL-6 in the mouse skin after laser exposure has been demonstrated in the immunological assay, whereas no change in the TNF- α level compared to the unirradiated control animals has been registered. Thus, laser irradiation at the wavelength of 1270 nm and energy density of 1.2 J/cm² causes the changes in the redox potential of cells, synthesis of signaling molecules

(cytokines and miRNAs) triggering protective reactions: increase in antioxidant properties of red blood cells, decrease of proinflammatory cytokine synthesis. So, we may conclude that this PBM regime induces both local and systemic biostimulating effects, presumably due to the short-duration increase of ROS levels which turns active the cell antioxidative system.

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Keywords

BALB/c Nude mice, photobiomodulation, low-level lasers, 1270 nm, cell proliferation, cell antioxidant system

1. Introduction

For the last decades, laser systems have been enthusiastically studied and then used in different ways in industry, telecommunication, and biomedicine. One of fields where lasers have found very broad applications is biology and medicine utilizing them for microscopy, flow cytometry and in photodynamic therapy and diagnostics. Also the lasers are widely used in the wound healing, and recreational medicine, so-called photobiomodulation (PBM), due to the absence of side effects, pain, and precise control of the irradiation dose. Some PBM effects such as photoinduced dissociation of oxyhemoglobin (HbO_2), where molecular oxygen is released [1], light-oxygen and photodynamic effects related to the singlet oxygen formation [2], and biotissue heating associated with absorption of radiation and nonradiative energy loss in hemoglobin molecules [3] have been observed in cells after employing PBM at the wavelengths of 585-587 nm, 632 nm, 950-960 nm, and 1267-1270 nm. These processes are determined by the PBM wavelength and radiation energy density applied. The strongest resonance absorption band of oxygen is in the range of 1265-1270 nm, where no absorption of known chromophores occurs in the living cell. The light-oxygen effect (1265-1270 nm ranges) can activate or damage (depending on the energy density) the biosystems through a direct photoexcitation of molecular triplet oxygen into the singlet oxygen [4–8].

It has been demonstrated that in cells with no intrinsic photoreceptors laser photodynamic effects may activate through the oxidative stress gene expression and synthesis of enzyme cascade responsible for the cell protective responses [9–11]. These explain some biological and therapeutic effects induced by PBM in the visible spectrum range on cells and tissues with no exogenous dyes loaded in [12–14]. It has been found that laser irradiation at the wavelength within the infrared range (IR) at a dose up to 30 J/cm^2 activates microcirculation, whereas at a dose higher than 70 J/cm^2 may in contrary disorder microcirculation [15]. Irradiation in the energy density range of $0.005\text{--}0.05 \text{ J/cm}^2$, $0.1\text{--}1.0 \text{ J/cm}^2$, $0.1\text{--}3.0 \text{ J/cm}^2$ stimulates cell proliferation, tissue metabolism and organ functioning, and analgesia, correspondently [16].

IR PBM effects on skin are deployed in cosmetology and wounds healing through antimicrobial, anti-inflammatory and regenerative effects [17]. Hypothesizing that the extensive number of the different skin receptors related to different organs can be the points through which the PBM has its systemic effects on organism. As such the PBM absorption depends on the tissue properties. At a wavelength of $0.6\text{--}1.4 \mu\text{m}$ and over, the skin, muscles and bones, parenchymal organs and highly vascularized tissue absorb 25-40%, 30-80%, and

up to 100% of radiation, respectively [18]. IR laser radiation at the doses of 5 - 40 J/cm² are often used for local laser hyperthermia of neoplasms inducing coagulative necrosis of the tumor with minimal damage to surrounding healthy tissues [19].

Laser irradiation effects at the wavelength within the resonance absorption band of molecular oxygen (1270 nm) on biochemical and physiological processes in Balb/c Nude mice are of particular interest of the medics and physiologists due to their unknown nature (mechanisms) related to the advantages of this wavelength irradiation in the tumor phototreatment and organism photobiomodulation [20–23]. This research will focus on revealing the mechanisms of non-thermal low intensity near infrared irradiation effects *in vivo*. Therefore, we propose a comprehensive biomolecular analysis of the changes in the BALB/c Nude mice skin after an exposure to the continuous irradiation at the 1270 nm wavelength.

2. Materials and Methods

Immunodeficient BALB/c Nude mice ("Pushchino" Ltd., Russia) were selected for control and experimental groups as an object of the study. The skin of these mice does not require hair removing, thus eliminating risk of additional skin irritation. Two experimental groups include animals (n = 8) irradiated by Raman laser operating at the wavelength of 1270 nm and power of 1.96 mW. Animals were exposed to a single irradiation session for 2 min at the intensity of 10 mW/cm² and energy density (ED) of 1.2 J/cm², and for 12 seconds at the intensity of 10 mW/cm² and ED of 0.12 J/cm².

The next day after irradiation, the animals from control and experimental groups were taken under ether anesthesia. The blood samples were taken and heparin-stabilized animal blood was used for further biochemical and immunological studies. Skin sample with a spot diameter of 0.5 cm exposed to laser irradiation on the lateral surface of the body was studied. The same skin area was explored in the control group animals. Unirradiated skin segments of control animals and irradiated skin regions of experimental animals were fixed in 10% neutral formalin for histological examination and in RNA-later solution for further studies on microRNA expression. All experiments were performed in accordance with World Medical Association Declaration of Helsinki (1964) amended in 1975, 1983 and 1989 and Ethical Approval Committee of Ulyanovsk State University (No. 9, September 15, 2017).

Morphological methods

The irradiated skin patch of 0.5 cm in diameter was taken for histological assessment. The biopsy material was fixed in 10% neutral formalin and series of skin sections were stained with hematoxylin-eosin. Morphometry of skin structures was carried out using a video-test system supplied with Motic microscope, JVC digital video camera (Japan), and density photometry software «Mekos-C1» (Russia) at 10 to 40 times magnification. The quantitative analysis was performed for a conventional unit of the sections. Morphometric data were recognized as statistically significant when P calculated by the two-sided Student t-test was <0.05.

Molecular genetics methods

The irradiated skin section 0.5 x 0.5 cm was fixed in the RNA-later solution (LLC "Eurogen", Russia) and then was cut using sterile tweezers for an isolation of total RNA employing the "Extraction 100" kit (LLC "Vector Best", Russia). The reverse transcription

reaction was launched immediately after the RNA isolation (set MMLVKit, LLC Eurogen, Russia) with miRprimer2 to design primers for the microRNAs examined. MicroRNAs miR-31, -130a, -191, -200c, -205 were used to investigate the expression pattern and its involvements in the skin physiological processes [24–32]. MicroRNA profile for the skin samples were evaluated with the CX96 nucleic acid PCR amplifier (BioRad, USA). To normalize the data, miRNA-191 was chosen as the skin-referred gene for skin cells. Calculation of the relative expression was performed according to the Qiagen protocol: $\Delta\Delta Ct = \Delta Ct1 \text{ (experiment)} - \Delta Ct2 \text{ (normal)}$.

Normalized target gene expression level = $2^{-\Delta\Delta Ct}$.

Biochemical methods

The level of lipid peroxidation products (diene conjugates (DC), ketodienes (KD), Schiff bases (SB), malondialdehyde (MDA)) and activity of the antioxidant system enzyme, catalase and reduced glutathione (GSH) level in the red blood cell hemolysate (1:10) were assessed. The DC, KD, conjugate trienes (CT) and SB levels were spectrophotometrically determined by Volchegorsky method [33] in heptane-isopropanol extracts. The concentrations of these products were calculated from the ratios between the optical densities of the heptane phase of E232/220 (DK), E278/220 (KD and CT), E400/E220 (SB) and were given in oxidation index units (O.I.U.). The MDA level ($\mu\text{mol/L}$) was determined in the test with thiobarbituric acid according to [34]. The catalase activity and GSH level were determined as referred [35].

Immunological methods

To assay the cytokine levels in the skin homogenate and blood plasma from the control and experimental animals, immunoenzyme kits were used to quantify the murine tumor necrosis factor (TNF- α) and interleukin-6 (IL-6) (eBioscience, USA). The statistical significance of the results was estimated using the nonparametric Mann-Whitney U-test. Differences between groups were considered significant at $p < 0.05$.

3. Results and Discussion

The laboratory BALB/c Nude mice used in the experiments are well known hairless and immunodeficient model which makes them suitable for research in dermatology, oncology, and immunobiology. Since the present research highlights the laser radiation effect on physiological processes in skin at the wavelength of 1270 nm, its results allow estimating laser potential for melanoma treating. So, the BALB/c Nude mice used as a model are quite suitable for the experiment.

I. The histological analysis has shown that the back skin of the control group animals has a fine-folded relief shape and thin epidermis.

Epidermis usually comprises 2-3 rows of grown cell layers. In the first row (basal layer), the basal cells with dark purple round nuclei are clearly visible (Fig. 1). The second row (thorny layer) comprises 1-2 rows of keratinocytes. Next granular layer includes one row of strongly flattened cells with uneasily distinguished nuclei. However, the dark-violet keratohyalin granules are easily observed in the cytoplasm. The stratum corneum is thin and tightly

adjacent to the granular layer peeling off in some places. A slight diffuse lymphoid-leukocyte infiltration is observed in dermis.

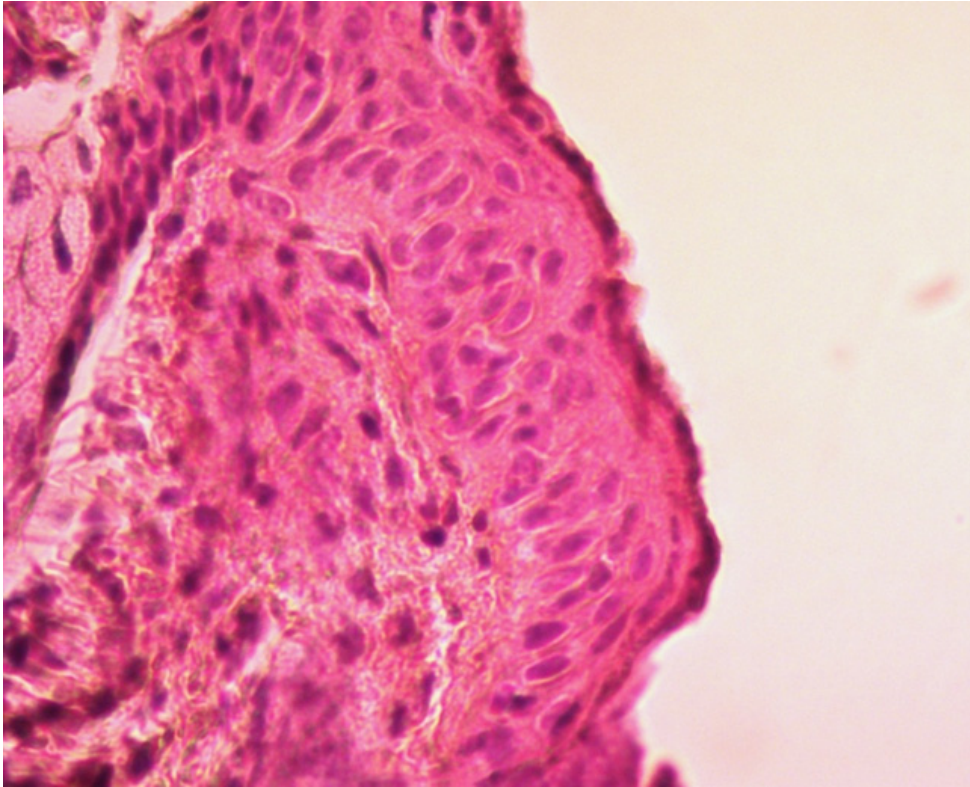


Figure 1. High-resolution histology image of Balb/c Nude mouse skin from the control group. Light microscopy (LM) x400. The sample is stained with hematoxylin-eosin.

The dermis papillary layer is smoothed and papillae are sparse. Loose fibrous unformed connective tissue located sub-epidermally consists of thin collagen fibers and transfers into reticular dermis layer with no sharp boundaries.

The reticular dermis layer is composed of a dense fibrous unformed connective tissue consisting of multidirectional thick collagen fibrils. The connective tissue cells are mainly fibroblasts, macrophages, tissue basophils and some other differentiated cellular elements.

Noteworthy, histological analysis of skin samples in the second experimental group irradiated at the wavelength of 1270 nm and $E = 0.12 \text{ J/cm}^2$ demonstrated no differences from the control group animals (Fig. 1).

After laser exposure at the wavelength of 1270 nm and $E = 1.2 \text{ J/cm}^2$ the mice skin has also a fine-folded relief and the skin structure is not changed (Fig. 2).

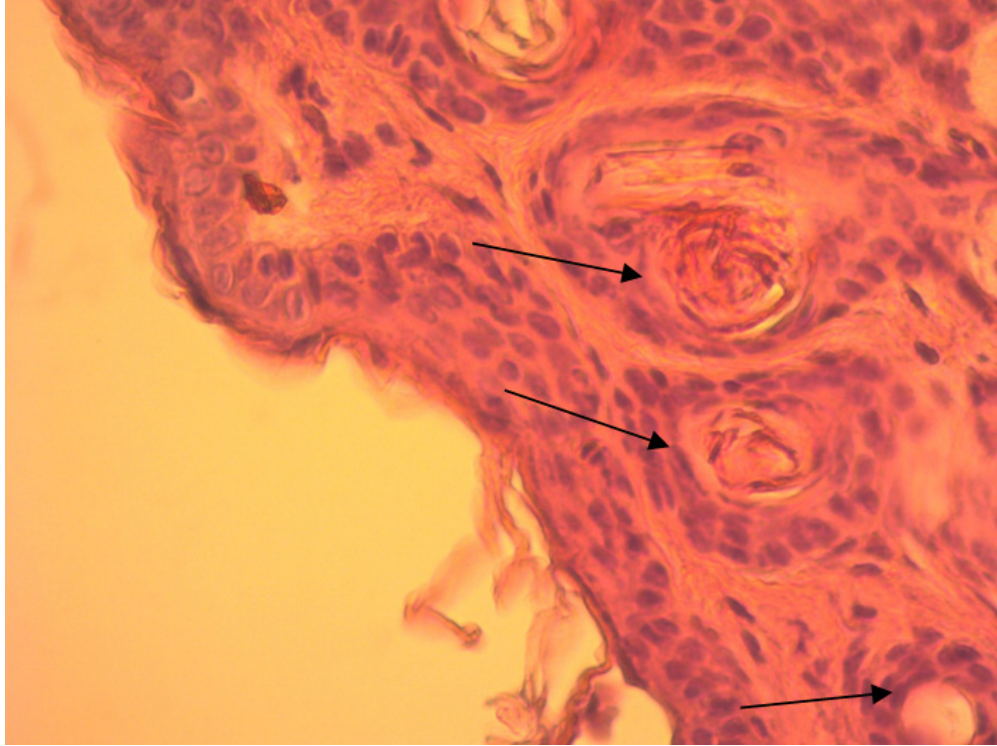


Figure 2. High-resolution histology image of Balb/c Nude mouse skin after a single exposure to 1270 nm laser irradiation at $E = 1.2 \text{ J/cm}^2$. LMx400. The sample is stained with hematoxylin-eosin (arrows point to the cells of epidermis growth layer).

The epidermis was not thickened over the normal size. The growth layer comprises 2-3 rows of keratinocytes with definably colored rounded nuclei which have clearly visible nucleoli. In some places, the growth layer cells are overlapping each other. In these regions, the nuclei are elongated. The granular layer and stratum corneum are kept unmodified. Like in the control group, the stratum corneum is thin with no signs of damage, peeling off, and vacuolization. The dermis papillary layer is not modified clearly indicating that the stratum corneum does not show any pathological signs. In the interlobular connective tissue of subcutaneous adipose tissue and in endomysium, perimysium (intermuscular connective tissue), moderate diffuse lymphoid-leukocyte infiltration was visualized.

In the first experimental group, the morphometric analysis reveals a significant proliferation of keratinocytes in the basal layer compared to the control group (Table 1). An increase in the number of basal epitheliocytes probably due to a higher level of cell mitotic activity in this layer after exposure to 1270 nm laser irradiation. In the material, there are few mitotic figures, since the experimental material is taken 24 hours after irradiation. Mitotic division

lasts some 6-8 hours. Thus, 24 hours after laser exposure more nuclei are observed per unit area.

Table 1 Number of basal epidermis layer cells per arbitrary unit area.

	Control group	First irradiated group, E=1.2 J/cm ²	Second irradiated group, E=0.12 J/cm ²
Average number of cells	7,22 ± 0,22	7,92 ± 0,26*	7,24±0,20

*- significant difference from the control value, $p < 0.05$.

In the second experimental group, no significant differences from the control group were registered.

As it was reported, the PBM experiments on dog epidermal keratinocytes, at the energy densities of 0.1, 0.2, 1.2 and 10 J/cm² (He-Ne laser, $\lambda = 650$ nm) stimulated the proliferative activity of skin cells. Therefore, these doses were chosen for experimental study on healing wounds in vivo models [18].

II. Experimental studies show the role of microRNAs in maintaining homeostasis of skin as an integral organ through their participation in regulation of proliferation, differentiation and apoptosis [15,24,25]. MicroRNAs are a relatively new class of endogenous, non-coding single-stranded small RNAs (18-22 nucleotides) participating in regulation of gene expression at the posttranscriptional level by inhibiting translation or destruction of specific transcripts of matrix RNA [26]. The pattern of microRNA expression has some tissue specificity. The expression pattern is studied for microRNAs, whose role in the physiological processes of skin has been demonstrated [28,36,37], namely, miR-31, -130a, -191, -200c, -205. For endogenous control, TaqMan® Advanced miRNA Assays User Guide (ThermoFisher Scientific, USA, Cat. #A25576) recommends microRNA-191 to be used.

MicroRNA-31 is a key regulator of the proliferation and migration of keratinocytes (dividing cells of basal layer), differentiation and hair growth [27]. Increased expression of mir-31 appeared to be in some skin diseases characterized by excessive proliferation of keratinocytes, e.g., psoriasis, chronic inflammatory skin disease, long-term wound healing [28,29], and squamous cell carcinoma [30].

It has been demonstrated that miR-130a level increases in chronic wounds and delayed reepithelization [24]. Both MicroRNAs-200c and -205 participate in regulation of the cell cycle [31], as well as in the epithelial-mesenchymal transition and cell differentiation to the embryonic phenotype through direct (miRNA-200c) and intermediary (miRNA-205) regulation of ZEB1 and ZEB2 gene expression which suppresses E-cadherin gene transcription [32]. Thus, these miRNAs were chosen to study the genetic mechanisms involved in regulating the response to PBM 1270 nm.

Real-time PCR is used to assay miRNA expression in irradiated skin areas and intact area from the opposite side after laser exposure at $E = 1.2$ J/cm². Fig. 3 shows the expression of miR-31, -200c in skin after laser exposure.

In all skin samples irradiated with 1270 nm laser ($E=1.2$ J/cm²) a significant increase in miR-31 expression has been observed (Fig. 3), whereas microRNA-200c do not demonstrate such

an overall change. Expression of these genes has only been observed in two animals (No.1, 4) in the experimental group. However, in most cases (75%), the miR-200c expression went down. The data are calculated for endogenous control of miR-191 and are given in relative units to compare expression dynamics in the irradiated and non-irradiated skin region of the same mouse.

No significant changes in the expression profile of miR-205 and miR-130a were observed in any sample.

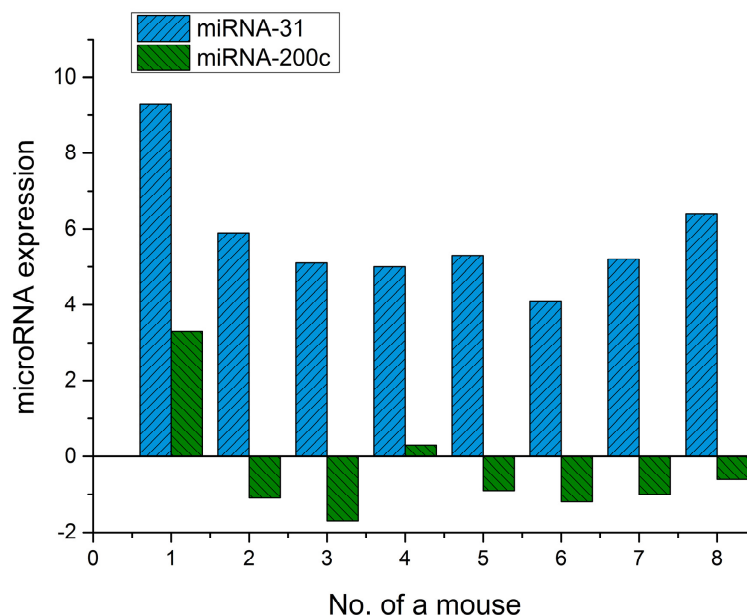


Figure 3. Expression of miR-31 and miR-200c in mouse skin after irradiation at 1270 nm (Log2 value $2^{-\Delta\Delta C_t}$).

Thus, only two miRNAs (miR-31 and -200c) change in the first experimental group. These post-transcriptional activity gene regulators control keratinocyte division and differentiation of other cell types. The changes registered in the skin of BALB/c Nude mice after 1270 nm laser applied with ED of 1.2 J/cm² indicates a biostimulating effect of PBM associated with an increase in proliferative activity of the basal layer cells in epidermis and posttranscriptional factor of microRNA-31 regulating this process in the skin.

In the second experimental group, there are no changes in miRNA expression.

III. Biochemical studies on blood hemolysate of BALB/c Nude mice show that PBM of 1270 nm and E = 1.2 J/cm² leads to a significant increase of the reduced glutathione level (GSH) in erythrocytes of irradiated mice (Table 2), whereas the level of primary lipid oxidation products (diene conjugates) increases slightly and the levels of ketodienes and Schiff bases in contrast demonstrate significant decrease (Table 3). Among the products of protein oxidative modifications, the level of primary ketone groups (530 nm) decrease significantly in mouse

erythrocytes (Table 4). The obtained data assume the increase in the antioxidant status of mice red blood cells under PBM of 1.2 J/cm².

Table 2. Activity of catalase and GSH in the erythrocytes of BALB/c Nude mice after PBM at 0.12 and 1.2 J/cm².

	Control, (n=7)	E=0.12 J/cm ² , (n=8)	E=1.2 J/cm ² , (n=8)
Catalase, mmol/min/l	6245.0±97.7	6246.1±164.2	6241.4±235.6
GSH, μmol/l	0.92±0.066	0.99±0.025	1.35±0.074*

* - the data are significantly different from the control group results.

Table 3. Level of lipid peroxidation products in erythrocytes of BALB/c Nude mouse after PBM at 0.12 and 1.2 J/cm².

	Control, (n=7)	E=0.12 J/cm ² , (n=8)	E=1.2 J/cm ² , (n=8)
MDA, μmol / l	570.9±24.4	572.4±31.8	583.0±39.9
DC, (heptane phase), units of oxidation index 232/220	1.053±0.011	1.048±0.015	1.119±0.008*
KD, (heptane phase), units of oxidation index 278/220	0.173±0.003	0.170±0.006	0.105±0.007*
SB, (heptane phase), units of oxidation index 400/220	0.050±0.004	0.049±0.002	0.021±0.003*

* - the data are significantly different from the control group results.

Table 4. Protein oxidative modification in erythrocytes after PBM at 0.12 and 1.2 J/cm².

	Control, (n=7)	E=0.12 J/cm ² , (n=8)	E=1.2 J/cm ² , (n=8)
Neutral aldehyde groups	2.908±0.193	2.884±0.107	2.375±0.056*
Neutral ketone groups	3.412±0.222	3.452±0.149	2.780±0.054*
Primary aldehyde groups	2.382±0.199	2.307±0.126	1.850±0.069*
Primary ketone groups	0.752±0.079	0.721±0.093	0.558±0.025*

* - the data are significantly different from the control group results.

This irradiation regime (1.2 J/cm²) leads to protection of the membrane proteins and lipids, decrease in the pro-oxidant effect due to activation of antioxidant mechanisms, in particular, the endogenous glutathione system. The observed pronounced increase in GSH, as the main red blood cell antioxidant, could indicate an increase in its synthesis by glutathione-dependent enzymes and through its participation in utilization of pro-oxidants. The most pronounced changes are observed in the level of oxidative modification of membrane proteins (Table 4). It can indicate a greater sensitivity of protein molecules to PBM at the wavelength

of 1270 nm. Twenty-four hours after laser exposure, detoxification of reactive oxygen species intensifies that could indirectly indicate an increase of the singlet oxygen generation in response to laser exposure.

Thus, PBM causes an activation of the endogenous antioxidant defense systems and suppresses the action of reactive oxygen species generated in BALB/c Nude mice red blood cells immediately after laser exposure. This effect is consistent with the reported data highlighting the PBM-induced membrane-stabilizing effect and enhanced antioxidant blood properties reported earlier [38-40]. Thus, PBM stimulates the protective effect in red blood cells even at short exposure time and low irradiation dose (1.2 J/cm^2).

IV. The cytokines usually act through interaction with specific receptors at the plasma membrane rafts, which are expressed in small amounts on the cells [41]. They regulate the activation, differentiation and proliferation of immunocompetent and somatic cells. These regulatory proteins are involved in modulation both inflammatory and immune responses and are critical elements in the skin response to damage or viral-infection. IL-6 belongs to potent pro-inflammatory cytokines that stimulates B-cell proliferation and differentiation. It is involved in the production of multipotent colony-forming factors and megakaryocytes. IL-6 is found in normal-skin fibroblasts and sweat ducts [42]. It is shown that IL-6 is secreted by almost all cells, including keratinocytes, melanocytes and Langerhans cells after their stimulation with various factors, including cytokines, tumor necrosis factor (TNF), platelet-derived growth factor (PDGF), bacterial and virus infection, and microbial lipopolysaccharides (LPS).

TNF- α is produced by monocytes, macrophages, T cells (Th1), keratinocytes, neutrophils, natural killers, endothelial, mast and myeloid cells, and lymphokine-activated killer (LAK) cells playing an important physiological role in immunoregulation [43]. TNF- α stimulates lymphocytes, neutrophils, eosinophils, endothelial cells, fibroblasts, inducing the production of IL-6 in many cell types [44]. However, it has been shown that TNF- α does not affect proliferation and differentiation of normal keratinocytes in a skin model [45]. TNF- α participates in inflammation and its level increases with the inflammation progression [46]. The experimental data report that PBM decreases production of IL-6 and TNF- α in the supernatant of activated mononuclear cells [47,48].

Twenty-four hours after exposure to a diode laser at 780 and 660 nm, in brain and blood tissues, a decrease in the level of IL-6 and TNF- α has been observed [49]. When analyzing the immunomodulatory PBM effect on the skin contact hypersensitivity reaction, no significant changes have been registered in TNF- α concentration [50]. Despite the progress achieved in studying the mechanisms of immunogenesis regulation, the immunomodulatory effect of laser radiation in vivo on the pro-inflammatory cytokine synthesis is still unclear.

The analysis of the results shows that the level of IL-6 in skin irradiated with $\lambda = 1270 \text{ nm}$ is significantly lower than its level in the non-irradiated area of the BALB/c Nude mouse skin, whereas TNF- α level increases slightly in response to laser exposure (Table 5). No significant differences are observed in the animal group irradiated at $E = 0.12 \text{ J/cm}^2$.

Table 5. Level of cytokines in homogenate of irradiated BALB/c Nude mouse skin

	Control, (n=7)	E=0.12 J/cm ² , (n=8)	E=1.2 J/cm ² , (n=8)

IL-6, pg/ml/mg protein	3499.198 (1610.85 – 6916.44)	3346.673 (1390.62 – 5816.57)	2446.90* (1674.28 – 3145.37)
TNF-α, pg/ml/mg protein	2652.553 (961.54 – 4000)	2599.464 (905.68 – 4000)	2839.86 (1363.64 – 4000)

*The data are significantly different from the control group results ($p < 0.05$).

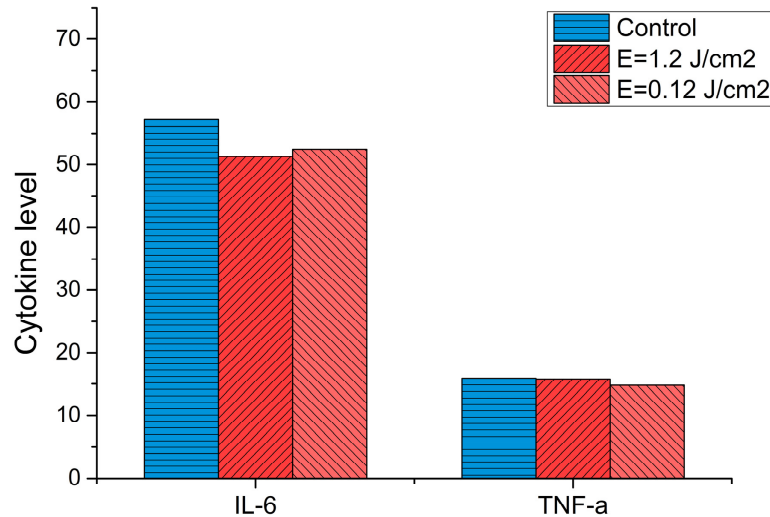


Figure.5. Cytokine level in the blood plasma of BALB/c Nude mouse after laser exposure.

In the first experimental group the level of IL-6 decreases in blood plasma (51.26 ± 14.45 pg/ml versus 57.14 ± 12.82 pg/ml in the control group). TNF- α level in the blood plasma was the same in both experimental and control groups (Fig. 4). This is a positive dynamic, since laser radiation being 1.2 J/cm^2 does not induce the inflammatory reaction in the skin and the blood. Moreover, laser radiation reduces to some extent production of pro-inflammatory cytokines, in particularly, interleukin-6.

4. Conclusion

In the BALB/c Nude mouse model, PBM has been shown to induce a photo-stimulating effect of the 1270 nm radiation at the dose of 1.2 J/cm^2 in both an irradiated skin spot and peripheral blood cells. This effect is associated with biochemical reactions changing cell redox state, synthesis of signal molecules (cytokines and microRNAs) and also responsible for consequent physiological effects. In this case, the laser triggers cell protective effects through direct excitation of oxygen molecules. Nonetheless, low intensity and short exposure time of laser radiation cause pronounced effects on the membrane-protective action and enhanced antioxidant system. Also this irradiation decreases the synthesis of pro-inflammatory cytokines in blood plasma, mainly IL-6. Locally, in the region of laser

exposure, the proliferative processes intensify with no signs of inflammation. Thus, in the BALB/c Nude mouse model the laser at the wavelength of 1270 nm and dose of 1.2 J/cm² produces local and systemic stimulation effect highly likely due to a short-term increase in the ROS levels which turns active the cell antioxidative system.

The further studies should be done with higher radiation doses for revealing whether 1270 nm radiation can treat the melanoma planted on BALB/c Nude mouse model.

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