EFFECT OF PHOTOBIOMODULATION THERAPY ON THE PROCESSES OF REPAIR OF COMPLEX WOUNDS IN AN EXPERIMENTAL STUDY
VPLYV FOTOBIOMODULAČNEJ TERAPIE NA PROCESY HOJENIA KOMPLEXNÝCH RÁN V EXPERIMENTÁLNEJ ŠTÚDII

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ABSTRACT
Introduction: The treatment of complicated wounds is a continuously developing area of medicine. Photobiomodulation (PBM) has become widespread type of physiotherapy for many diseases, including soft tissue injuries. The mechanisms underlying PBM are still not fully understood.
Our work aimed to study the utility of interleukin 8 (IL-8), platelet growth factor (PDGF), and vascular endothelial growth factor (VEGF) in the regulation of reparative processes in complicated wounds under conditions of local hypoxia using PBM therapy of various parameters in the experiment.
Materials and methods: Complex wounds were simulated in rats. Animals in the experimental groups were exposed to PBM therapy of various parameters. The levels of IL-8, PDGF, and VEGF were studied by enzyme immunoassay in blood serum. Histological studies of wound samples were carried out.
Results: The use of PBM therapy increases PDGF levels (control of proliferation), as well as modulation of IL-8 and VEGF expression (control of inflammation and angiogenesis). Histological examination showed faster maturation of granulation tissue in groups of animals whose wounds were exposed to PBM therapy with the parameters used in the study, which corresponds to an earlier transition of the process to the remodeling phase.
Conclusions: Proper selection of parameters of PBM therapy can make it possible to create a tool to compensate for changes that lead to disruption of reparative processes, thereby contribute to the optimization of processes in the healing of complicated wounds.

Key words: Complex wound. Photobiomodulation therapy. Experimental model. Growth factors.

ABSTRAKT
Úvod: Liečba komplikovaných rán je neustále sa rozvíjajúcou oblasťou medicíny. Fotobiomodulácia sa rozšírila ako typ fyzioterapie pri mnohých ochoreniach, vrátane poranení mäkkých tkanív. Mechanizmy, ktoré sú základom fotobiomodulácie, stále nie sú úplne pochopené.
Cieľom našej práce bolo študovať úlohu interleukínu 8 (IL-8), rastového faktora krvných doštičiek (PDGF) a vaskulárneho endotelového rastového faktora (VEGF) v regulácii reparačných procesov v komplikovaných ranách v podmienkach lokálnej hypoxie pomocou fotobiomodulácie (FBM) terapie rôznych parametrov v experimente.
Materiály a metódy: Komplikované rany boli simulované u potkanov. Zvieratá z experimentálnych skupín boli vystavené FBM terapii rôznych parametrov. Štúdium hladín IL-8, PDGF a VEGF sa uskutočnilo enzýmovým imunotestom v krvnom sére. Uskutočnili sa histologické štúdie vzoriek rán.
Výsledky: Použitie FBM terapie viedlo k zvýšeniu hladín PDGF (kontrola proliferácie), ako aj k modulácii expresie IL-8 a VEGF (kontrola zápalu a angiogenézy). Histologické vyšetrenie ukázalo rýchlejšie dozrievanie granulačného tkaniva u skupín zvierat, ktorých rany boli vystavené FBM terapii s parametrami použitými v štúdii, čo zodpovedá skoršiemu prechodu procesu hojenia rany do fázy remodelácie.
Záver: Správny výber parametrov FBM terapie môže umožniť vytvorenie nástroja na kompenzáciu zmien, ktoré vedú k narušeniu reparačných procesov, a tým prispieť k optimalizácii procesov pri hojení komplikovaných rán.

Kľúčové slová: Komplexná rana. Fotobiomodulačná terapia. Experimentálny model. Rastové faktory.

INTRODUCTION

Wound healing is one of the most complex processes in multicellular organisms, involving numerous intra- and intercellular signaling pathways in various cell types [1]. The wound healing process consists of four well-organized and largely overlapping phases: hemostasis, inflammation, proliferation, and remodeling. Growth factors, cytokines, and low molecular weight serum proteins regulate cellular responses and stimulate intracellular signaling pathways involved in the regulation of cell proliferation, differentiation, migration, and protein synthesis. Such pathways are activated in response to a wide range of extracellular signals. Violating the interaction of signaling pathways, strictly coordinated in time and space, leads to complicated and long-term non-healing wounds.

The treatment of complicated wounds is a continuously developing area of medicine. Currently, along with the methods used, such as treatment of wounds with negative pressure, the use of ultrasound, magnetic surgical instruments, growth factors, natural sources of bioactive compounds, etc. [2-6], the search for new methods of wound debridement and stimulation of the reparative process continues.

Because of injuries, there are changes in the relationship between the regulation of the neurohumoral and immune systems [7]. The need for rehabilitation measures in the recovery process of traumatic injuries of the neuromuscular apparatus is shown [8].

An innovative strategy for the treatment of a wide range of neurological conditions is photobiomodulation (PBM) using light in the red or near-infrared range [9]. PBM is also used to treat dental patients. Laser irradiation is effective when exposed to wound-healing sites after tooth extraction and bone regeneration [10]. The effectiveness of PBM in the treatment of pulmonary diseases [11] and the reduction of acute and chronic musculoskeletal pain [12] has been demonstrated. Whole-body PBM treatment offers the possibility of both central and peripheral intervention, resulting in a systemic response [13].

Thus, PBM therapy is a non-invasive method that helps reduce inflammation while accelerating healing and tissue repair processes [14]. Photobiomodulation promotes angiogenesis during wound healing [15]. The important role of PBM in the regulation of numerous signaling pathways involved in wound healing has been shown [16]. The use of PBM therapy allows the body to regulate the subtleties of recovery processes by modulating intercellular mediators [17]. When choosing the parameters of PBM therapy, it is necessary to consider the dose-dependent effect of laser radiation – the law of the two-phase dose response of Arndt-Schulz [18].

Despite the widespread use of this method and the positive results obtained, the mechanisms underlying PBM are still not fully understood.

Our work aimed to study the role of interleukin 8 (IL-8), platelet growth factor (PDGF), and vascular endothelial growth factor (VEGF) in the regulation of reparative processes in complicated wounds under conditions of local hypoxia using PBM therapy of various parameters in the experiment.

MATERIALS AND METHODS

Animals

24 Wistar rats weighing 250 ± 30 g at the age of 9 months were involved in the experiment. The experiments were carried out by the principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986). The study was approved by the Committee on Ethical Animal Care and Use of the Kharkiv Medical Academy of Postgraduate Education, Ukraine. All animal handling procedures were performed in accordance with the rules and guidelines set by this Committee. All operations were performed under general anesthesia (zoletil mononarcosis 10 mg/kg body weight) and every effort was made to minimize animal suffering.

Experimental protocol

Animals were randomly divided into 4 groups of 6 rats each was used during the experiment. Intact animals (Int): rats in this group had no wounds on their skins. Complicated wounds in the proximal back of rats were simulated in 18 rats (experimental and control groups). With the help of pointed scissors, a skin flap with a diameter of 2 cm was removed. After that, a perpendicular loop-shaped skin-fascial suture was applied along the edges of the wound. On the surface of the bottom of the wound, the superficial fascia was dissected by perpendicular incisions with the formation of cells 5 × 5 mm in size, which were sutured with U-shaped sutures [19].

Parameters of PBM therapy

The wounds of the animals of the experimental groups were exposed to PBM therapy. The wounds of animals in the control group (Con) were irradiated fictitiously. Rats of the experimental groups received PBM therapy once a day for 5 days, starting 24 hours after the formation of the wound. The laser device “Lika-therapist M” (Ukraine) was used in continuous mode at 660 nm wavelengths and an energy density of 1 J/cm^2. In the first experimental group (Exp 50), the output power was 50 mW, and in the second (Exp 10), 10 mW. The distant method was used to avoid contact with an open wound. Operators wore safety goggles during treatment to avoid possible eye damage.

Blood Collection

The animals were taken out of the experiment on the 14th day. Animals were euthanized by inhalation of chloroform in a confined space. Blood samples were obtained by open heart puncture. The levels of IL-8, PDGF, and VEGF were studied by enzyme immunoassay in blood serum per the manufacturer's instructions. IL-8 and VEGF levels were determined using Vector-Best reagent kits (Ukraine). The PDGF level was determined using the eBioscience kit (USA).

Histological evaluation

For histological examination, a section of the wound was cut out, including all its departments (central, main, edge). The material was embedded in paraffin and stained with picrofuchsin according to Van Gieson according to the standard method. The preparations were analyzed and photographed using a PrimoStar microscope (Zeiss, German) and a Microocular digital camera.

Statistical analysis

Statistical processing of the results was performed using Statistica 6.0 (StatSoft, USA) statistical analysis package. The significance of differences between groups (statistical significance) was determined using the non-parametric Kruskal-Wallis ANOVA test for independent samples. The significance $p < 0.05$ was considered statistically significant. The descriptive data were expressed as M ± SE, where M is the arithmetic mean, and SE is the standard error of the arithmetic mean. Histograms were plotted using the GraphPad Prism 7 package (GraphPad Software, USA).

RESULTS

The concentrations of IL-8 and growth factors in the blood serum of animals are shown in Fig. 1 – 3.

The effect of PBM on the expression of IL-8 and growth factors in the serum of animals with complicated wounds demonstrated: There were no differences in the experimental groups in the levels of VEGF and IL-8 compared with those of the control group, while the concentrations of VEGF and IL-8 in experimental group 2 (Exp 10) were lower compared to those in experimental group 1 (Exp 50) ($p < 0.05$) (Fig. 1–2); an increase in PDGF levels 14 days after wound modeling in experimental groups compared with the control group ($p < 0.05$) (Fig. 3).

The histological examination of wound samples on the 14th day of the experiment in animals of all groups showed incomplete epithelialization of the wound, its central part remained covered with a scab. In different areas, both a multilayered epidermis, with a clear differentiation into layers, and a thinned epidermis, consisting of 1-3 layers of flat cells, were observed. In the marginal sections of the wound, newly formed hair follicles and sebaceous glands were located (Fig. 4).

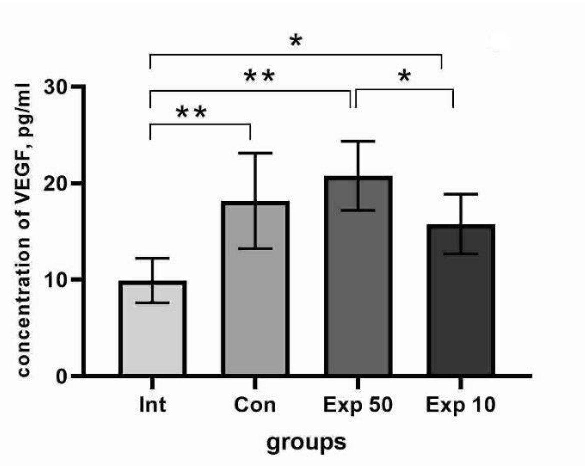

Figure 1 Changing the levels of VEGF in the blood serum of animals on the 14th day (*$p < 0.05$, **$p < 0.001$)

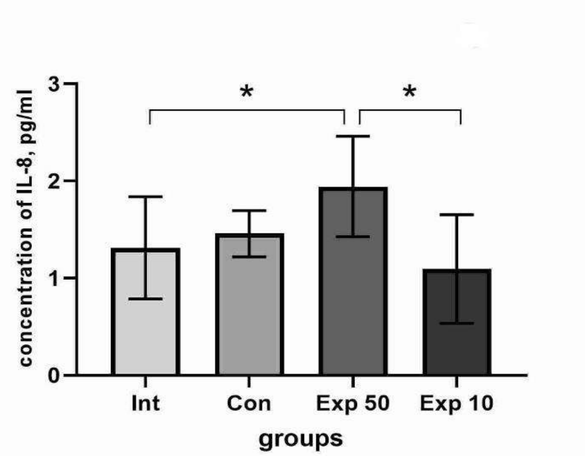

Figure 2 Changing the levels of IL-8 in the blood serum of animals on the 14th day (*$p < 0.05$, **$p < 0.001$)

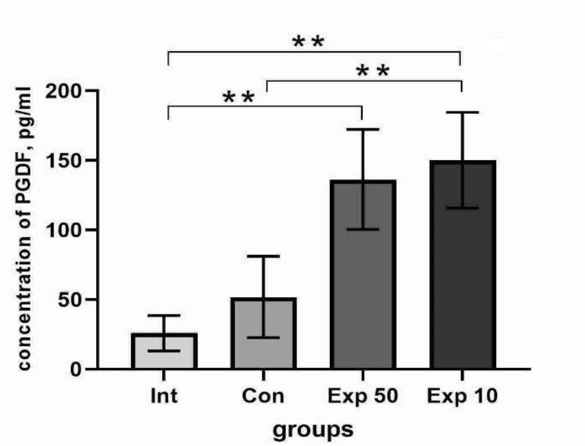

Figure 3 Changing the levels of PDGF in the blood serum of animals on the 14th day (*$p < 0.05$, **$p < 0.001$)

In animals of the control group, the wound cavity was filled mainly with maturing connective tissue with a moderate number of vessels and single neutrophils, lymphocytes, and macrophages. Central areas were noted, consisting of young granulation tissue with thin-walled capillaries and vascular cavities, foci of inflammation, and hemorrhages (Fig. 4a).

In the experimental groups of animals were observed in the areas of the wound adjacent to healthy skin, mature connective tissue with densely packed bundles of collagen fibers of sufficient thickness, few vessels, fibroblasts, and fibrocytes. The main part of the wound consisted of maturing connective tissue with densely packed bundles of collagen fibers oriented parallel to the wound surface (corresponding to mechanical load). The number of blood vessels differed in the experimental groups. In experimental group 1, the capillaries were noted in greater numbers and were often dilated and plethoric, often with perivascular hemorrhages, especially in the upper layers of the wound cavity (Fig. 4b). In areas near the bottom of the wound, a few vessels with a thickened connective tissue layer of the wall were observed. In experimental group 2, the number of vessels was smaller, and a few of them were dilated, without signs of plethora and hemorrhages (Fig. 4c).

Histological examination showed the faster maturation of granulation tissue in the experimental groups.

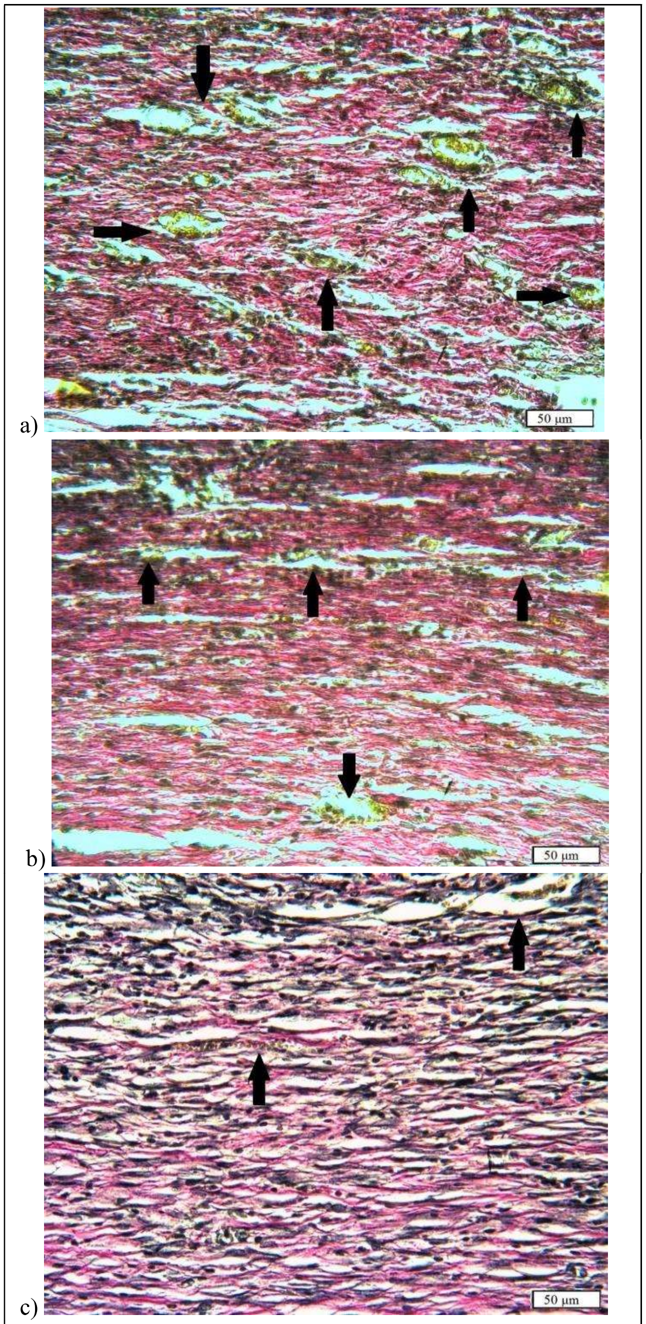

Figure 4 Wound areas from the central sections in rats after 14 days: a) in the control group – young granulation tissue with a large number of full-blooded capillaries (arrows), fibroblast proliferation, and chaotically arranged collagen fibers; b) in experimental group 1 – maturing granulation tissue with parallel oriented bundles of collagen fibers and a moderate number of vessels (arrows); c) in experimental group 2 – maturing granulation tissue with parallel oriented bundles of collagen fibers and single vessels (arrows). Van Gieson. Magnification 400x.

DISCUSSION

In our study, we studied the effect of PBM therapy on wound healing under experimentally created conditions of local hypoxia. Local tissue hypoxia is known to impair wound healing [20].

We studied the effect of PBM therapy on the expression of regulatory proteins that mediate important signaling pathways and act on the processes of cell regeneration and repair at the stage of transition from the proliferative phase to the remodeling phase. The studies of intercellular mediators at this stage of wound healing are rare. The proliferative phase of wound healing is characterized by granulation tissue formation, angiogenesis, wound contraction, and epithelization. The final phase of remodeling is characterized by the restructuring of the extracellular matrix, which can lead to scar formation. Angiogenesis is significantly enhanced by vascular endothelial growth factor VEGF [21]. VEGF also affects wound repair and closure, and granulation tissue formation [22]. In our study, when using

PMB therapy, there were no differences in VEGF expression during the healing of complicated wounds in the experimental groups. At the same time, in experimental group 2, the levels of VEGF decreased compared to the levels of VEGF in experimental group 1. Which, apparently, indicates an earlier transition to the remodeling stage in this experimental group. The decrease in the number of vessels in experimental group 2 was confirmed by the results of histological studies.

The findings are consistent with the study by Otterço et al., in which, in skin wound healing in rats, a statistically significant difference in VEGF increase was observed at day 11 in the PBM-treated group compared to the untreated group [23]. On day 16, there were no differences in VEGF levels between the study groups. However, our data contradict the results of a study in which irradiation of a burn wound with an InGaP-670 nm laser (9.86 J/cm^2) in an animal model resulted in an increase in VEGF on days 10 and 18 (formation of granulation tissue and re-epithelialization), promoting healing [24]. According to the literature, PBM therapy increases VEGF expression during the first two weeks of treatment but decreases in the third week after the induction of the lesion [25].

An important role in wound healing is played by the platelet growth factor PDGF, which enhances the migration of macrophages and fibroblasts, and promotes the synthesis of collagen and proteoglycan [26]. The results obtained in our work demonstrate that PBM stimulates the production of PDGF at the stage of transition from the proliferative phase to the remodeling phase in both experimental groups, which may be due to the ability of PBM to stimulate the faster organization of the collagen scaffold. Histological studies confirm the better organization of collagen fibers in wound samples of animals from experimental groups. Similar results were obtained by Otterço et al., in which histopathological analysis of rat skin wound specimens showed increases in collagen at days 11 and 16 in the PBM-treated group of animals compared to the untreated group [23].

We also studied IL-8, which, as a member of the pro-inflammatory CXC cytokine family, is involved in the mediation of inflammatory responses, including angiogenesis, leukocyte degranulation, and cell migration [27]. The results obtained in our work demonstrate that on the 14^{th} day of the experiment, the level of the pro-inflammatory cytokine IL-8 did not change in the experimental groups with the use of PBM therapy. At the same time, in the second experimental group, the levels of IL-8 decreased compared to those in experimental group 1. It is known that the use of PBM therapy has an anti-inflammatory effect. And in the absence of inflammation, PBM can provide pro-inflammatory mediators that can help with tissue remodeling [28].

Few studies in the literature have studied the effect of PBM on the expression of IL-8 and PDGF in the proliferation phase – the transition of proliferation to the remodeling phase. In a study by Keskiner et al., no differences were found in the levels of IL-8 and PDGF-BB in the wound fluid of the palate on the 12th day after laser irradiation [29].

The difference between wound models and PBM therapy parameters makes it difficult to compare the results obtained. Further studies are needed to optimize the parameters of PBM therapy when using this method to improve the repair processes of complicated wounds.

The results of our study demonstrate the ability of PBM therapy to regulate endogenous levels of intercellular mediators. The correct selection of exposure parameters can allow the creation of a tool for correcting (compensating) changes that lead to the disruption of reparative processes. This contributes to the optimization of processes in the healing of complicated wounds, which ultimately leads to the formation of a structurally desirable tissue, and therefore to an increase in the likelihood of functionally complete wound healing.

CONCLUSIONS

PBM therapy can be used as an auxiliary means of correcting reparative processes in the treatment of complicated wounds. Thanks to the use of photobiomodulation therapy, it is possible to achieve high levels of PDGF (proliferation control), as well as modulation of the expression of IL-8 and VEGF (control of inflammation and angiogenesis), which, with the appropriate selection of parameters that are optimal at each stage of the reparative process, will contribute to the development of methods for optimizing healing complex wounds.

Histological examination showed faster maturation of granulation tissue in groups of animals whose wounds were exposed to PBM therapy with the parameters used in the study, which contributes to an earlier transition of the wound-healing process to the remodeling phase and increases the possibility of forming a full-fledged tissue.

Funding

The study is a fragment of scientific research work of Kharkiv National Medical University on the topic "Optimizing the treatment of combat trauma in conditions of purulent complications and further rehabilitation due to the improvement of connective tissue repair", state registration No. 0123U100381 (2023–2025). This study was funded by the Ministry of Health of Ukraine from the state budget.

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