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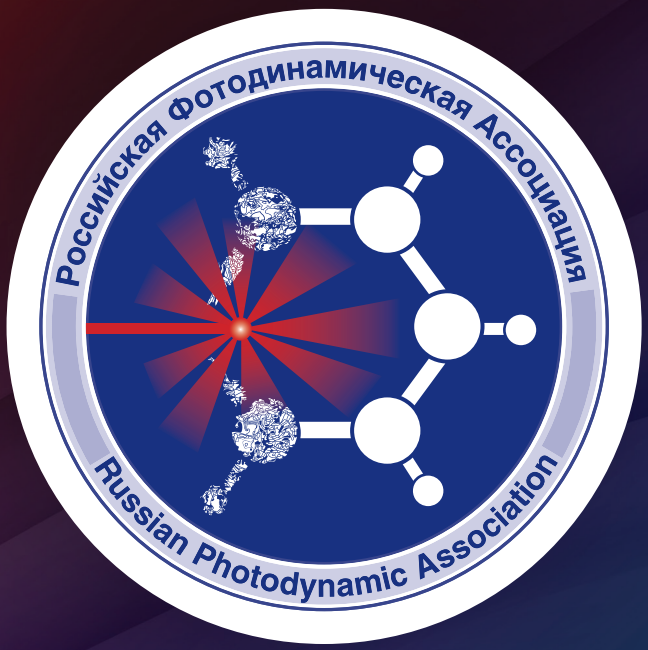

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ORIGINAL ARTICLES

ОРИГИНАЛЬНЫЕ СТАТЬИ

REVIEWS OF LITERATURE

ОБЗОРЫ ЛИТЕРАТУРЫ

ORIGINAL ARTICLES

EFFECTIVENESS OF 650 NM RED LASER PHOTOBIOMODULATION THERAPY TO ACCELERATE WOUND HEALING POST TOOTH EXTRACTION

Astuti S.D.[1], Nashichah R.[1], Widiyanti P.[1], Setiawatie E.M.[1], Amir M.S.[1], Apsari A.[2], Widyastuti[2], Hermanto E.[2], Susilo Y.[3], Yaqubi A.K.[1], Nurdin D.Z.I.[1], Anuar N.[4]
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Abstract

After tooth extraction, there can be consequences involving injury to the tissue surrounding the extracted tooth, which may lead to severe problems such as inflammation and infection. The wound healing process comprises inflammation, proliferation, and remodeling phases. Photobiomodulation is a therapy form that utilizes the interaction of a light source with tissue. This interaction can activate an increase in Adenosine Triphosphate (ATP), which subsequently triggers a chain reaction leading to the creation of new blood vessels and an increase in the number of fibroblasts. This study used a red laser light source with a power of 3.32 ± 0.01 mW, delivering a dose of 3.5 J to patients for extraction indications. The parameters observed included Interleukin 1β (IL-1β), Prostaglandin E2 (PGE2), Human Beta defensin 2 (HBD2), and Gingival Index (GI). The results of testing saliva samples using the enzyme-linked immunosorbent test (ELISA) for the parameters IL-1β, PGE2, and HBD2 show a significant influence between the control and therapy groups. Meanwhile, GI revealed a significant influence of therapy on the wound-healing process. Using the Mann-Whitney U test, on day 1, the p-value was found to be 0.32, indicating no significant difference between the control and therapy groups. However, on the third day after the therapy was administered, the p-value was obtained as 0.01, signifying a significant difference between the control and therapy groups. On day 5, a p-value of 0.034 was obtained, signifying a significant difference between the control and therapy groups. Based on the research results, it can be observed that there is a decrease in the values of IL-1β, PGE2, HBD2, and GI. This indicates that local immune cells, including resident macrophages, are activated by pro-inflammatory mediators released in response to injury, and they play an essential role in accelerating wound healing.

Key words: wound healing, red laser, photobiomodulation, enzyme-linked immunosorbent (ELISA), gingival index (GI).

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ЭФФЕКТИВНОСТЬ ПРИМЕНЕНИЯ ЛАЗЕРА С ДЛИНОЙ ВОЛНЫ 650 НМ ДЛЯ УСКОРЕНИЯ ЗАЖИВЛЕНИЯ РАН ПОСЛЕ УДАЛЕНИЯ ЗУБА

S.D. Astuti[1], R. Nashichah[1], P. Widiyanti[1], E.M. Setiawatie[1], M.S. Amir[1], A. Apsari[2], Widyastuti[2], E. Hermanto[2], Y. Susilo[3], A.K. Yaqubi[1], D.Z.I. Nurdin[1], N. Anuar[4]
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Резюме

После удаления зуба могут возникнуть осложнения, связанные с повреждением тканей, окружающих удаленный зуб, что может привести к серьезным проблемам, таким как воспаление и инфекция. Процесс заживления раны включает фазы воспаления, пролиферации и ремоделирования. Фотобиомодуляция — это форма терапии, которая использует взаимодействие источника света

с тканью. Это взаимодействие может способствовать выработке АТФ, что впоследствии запускает цепную реакцию, приводящую к образованию новых кровеносных сосудов и увеличению количества фибробластов. В нашем исследовании мы использовали лазер с излучением в красной области спектрв (мощность излучения 3,32 ± 0,01 мВт, световая доза 3,5 Дж). В ходе исследования оценивали влияние терапии на уровень интерлейкина 1β (IL-1β), простагландина E2 (PGE2), бета-дефенсина человека 2 (HBD2) и десневой индекс (GI). Исследования подтвердили значительное влиячние исследуемой терапии на процесс ранозаживления. По результатам исследований также наблюдали снижение значений IL-1β, PGE2, HBD2 и GI. Это указывает на то, что местные иммунные клетки, включая резидентные макрофаги, активируются провоспалительными медиаторами, высвобождаемыми в ответ на травму, и играют важную роль в ускорении заживления ран.

Ключевые слова: заживление ран, красный лазер, фотобиомодуляция, ферментный иммуносорбент (ИФА), десневой индекс.

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Introduction

Currently, 57% of Indonesians experience dental and oral health problems such as caries and periodontal tissue diseases. Periodontal disease is an ailment that affects the tissue surrounding and supporting the teeth, including the gingiva, periodontal ligament, cementum, and alveolar bone [1]. Periodontal disease occurs in developed and developing countries, affecting approximately 20-50% of the global population. The prevalence of periodontal disease in adolescents, adults, and older individuals makes it a public health problem [2]. Periodontal disease is a chronic inflammatory condition of the periodontium, and its advanced form is characterized by the loss of the periodontal ligament and the destruction of the surrounding alveolar bone. It is the leading cause of tooth loss and one of the two most significant threats to oral health [3].

Indications for teeth requiring extraction include severe caries, pulp necrosis, severe periodontal disease, orthodontic reasons, malpositioned teeth, cracked teeth, pre-prosthetic extraction, impacted teeth, supernumerary teeth, teeth associated with pathological lesions, pre-radiation therapy, teeth with jaw fractures, aesthetic considerations, and economic factors. Dental caries that involve the pulp tissue and leave only a tiny amount of healthy tissue make maintenance impossible. Severe periodontal disease causes bone resorption, resulting in tooth mobility, and extraction for orthodontic reasons may be necessary to create space. Malpositioned teeth can cause trauma to the surrounding soft tissues, and teeth with severe fractures may require extraction. Lastly, extractions may be performed for mouth preparation before prosthodontic treatment [4]. Tooth extraction, or simply extraction, is a procedure that involves separating a tooth from the soft tissue that surrounds it and removing a tooth that cannot be retained within its socket using forceps or an elevator. According to survey data, the Indonesian population's total number of teeth affected by decay reached 460 teeth per 100 people (4.6%), and 2.9% of cases of tooth decay required extraction [1]. In dentistry, tooth extraction is a procedure that can lead to injury, causing discomfort for the patient and increasing the risk of infections and other complications, which can result in more severe issues. Additionally, tooth extraction can traumatize blood vessels, initiating a primary hemostasis process that involves the formation of platelet plugs (blood clots) at the site of the wound. Platelet plugs form from interactions between platelets, coagulation factors, and the blood vessel walls [4]. Acute or chronic soft tissue injuries represent an abnormal condition for the patient and necessitate both time and financial resources for treatment. When tissue is damaged due to injury, a wound-healing process is initiated [5].

The wound healing process comprises three phases: the inflammatory phase, the proliferation phase, and the remodeling phase [6]. These phases are interconnected and overlap, beginning from when the wound occurs until healing and wound closure are achieved [7]. When an injury occurs, the body's initial response involves releasing platelets or blood clots, which contain hemostasis components. These platelet aggregates release Transforming Growth Factor beta 1 (TGF β1) as an inflammatory mediator, which activates fibroblasts to synthesize collagen [8]. Tissue healing following a wound is a complex process with several stages, influenced by numerous intrinsic and extrinsic factor [9,10].

The stages of healing are classified into three types: primary, secondary, and tertiary healing [11]. Various therapies are employed for wound healing, one of which is the use of antibiotics. Antibiotics can be pretty effective in treating infections and help prevent pain associated with wound-healing, even though the optimal dosage has not yet been determined. Antibiotics are commonly used to prevent infection during wound-healing, including wounds resulting from tooth extraction surgery. Doxycycline is an antibiotic frequently used for infections caused by gram-negative and gram-

positive microorganisms [12]. Since the correct dosage has not been established, and its use can potentially be inappropriate for the specific target or even inadvertently affect other areas due to the location of the wound in the mouth, alternative forms of treatment are needed to aid in the wound healing process following tooth extraction.

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used in periodontal disease therapy as anti-inflammatory drugs. These medications serve to alleviate pain and prevent the spread of inflammation by inhibiting the formation of prostaglandins through the cyclooxygenase (COX) pathway of arachidonic acid metabolism. However, prolonged use of COX-2 inhibitors can lead to adverse effects such as stomach ulcers and hemorrhage [13]. The side effects of non-steroidal anti-inflammatory drugs can encompass gastrointestinal disorders, cardiovascular diseases, and impaired kidney function. In patients with congestive heart failure, the use of non-steroidal anti-inflammatory drugs can exacerbate heart failure and pose risks to the gastrointestinal tract, including bleeding, ulceration, and perforation of the stomach or intestines, which can potentially be fatal. These side effects can manifest anytime during usage without warning symptoms. One issue related to drug usage is Adverse Drug Reactions (ADR) [14]. ADRs refer to adverse reactions to drugs that occur during clinical use. Non-steroidal anti-inflammatory drugs are among the medications most commonly associated with patients requiring treatment for ADRs [15]. Drugs that lead to ADRs are frequently encountered in high-risk patient populations [16]. Therefore, there is a need for alternative therapies that have fewer side effects than using these drugs. Photobiomodulation therapy is one treatment option associated with fewer side effects than drug usage [17,18].

Light sources utilized in photodynamic antimicrobial therapy and photobiomodulation encompass lasers [19] and LEDs [20,21]. Lasers emit coherent, collimated, monochromatic light. The effectiveness of laser therapy is influenced by the wavelength spectrum and energy density [22]. Laser therapy induces biochemical reactions in body tissues to facilitate cell repair, enhance blood circulation, and reduce inflammatory reactions and swelling. The energy delivered by the laser beam stimulates damaged cells to produce adenosine triphosphate (ATP), which is subsequently utilized to maintain normal cellular functions and promote cell repair [23].

Lasers at specific wavelengths have been demonstrated to inhibit the growth of bacteria [24,25] and biofilms [26-28], which are known to cause wound infections in vitro. Low-level laser therapy aims to reduce the toxicity of silver sulfadiazine and promote wound healing. In his literature, Walsh states that low-level laser therapy can impact wound healing [29]. Low-level laser therapy increases fibroblast proliferation, collagen synthesis, angiogenesis, and epithelialization. The laser stimulates the activation of the electron transport chain, ATP synthesis, and a reduction in cellular pH. This, in turn, triggers a reaction in the cell membrane through photophysical effects on calcium channels, resulting in an increased number of macrophages, along with enhanced fibroblast and lymphocyte cell activity [22,30].

Photobiomodulation (PBM), or low-level laser therapy, involves using red light to stimulate healing, alleviate pain, and reduce inflammation [18]. PBM is a therapeutic approach that leverages the interaction of a light source with tissue to trigger an increase in ATP, setting off a chain reaction that leads to the formation of new blood vessels and an increase in the number of fibroblasts responsible for generating a new matrix in injured tissue [31]. The mechanisms of photobiomodulation employ photons (light energy) to modulate biological processes.

PBM laser is also referred to as Low-level laser therapy (LLL), cold laser, therapeutic laser, and soft laser. This device utilizes laser energy, typically generated by a semiconductor diode with power levels ranging from 0.1 W to 0.5 W, for wound therapeutic purposes [2]. The primary chromophores involved are cytochrome c oxidase in mitochondria and calcium ion channels [32]. Secondary effects from photon absorption include increased ATP production, short bursts of reactive oxygen species, elevated nitric oxide levels, and modulation of calcium levels. Tertiary effects encompass the activation of various transcription factors, leading to increased cell survival, enhanced cell proliferation and migration, and the synthesis of new proteins. There is a biphasic dose-response relationship in which low light levels stimulate, while high light levels have an inhibitory effect. It has been discovered that PBM can induce the production of Reactive Oxygen Species (ROS) in normal cells. However, it reduces ROS levels when applied to cells under oxidative stress or in animal disease models. PBM can regulate antioxidant defences and decrease oxidative stress. Research has shown that PBM can activate NF-κB in normal quiescent cells, but in activated inflammatory cells, it reduces inflammatory markers [32]. Therefore, wavelength and dosage are crucial for effectively accelerating wound healing.

One of the benefits of PBM therapy is its ability to enhance the healing process of body tissue wounds. Injured body tissues often undergo a reduction or loss of their anatomical and functional structure. When this occurs, the body's natural mechanisms become crucial in restoring function and structure as part of the natural wound-healing process. To expedite the wound recovery process, a form of stimulation is required to encourage cells to enter the regeneration stage, and photobiomodulation therapy can play a significant role in this regard. As studied by researchers, the use

of low-power laser light sources in wound therapy has demonstrated a reduction in pain, a positive impact on inflammation, and facilitation of the proliferation and maturation phases, accompanied by an increase in tensile strength [33].

When lasers are applied to wounds, they induce changes in the permeability of inflammatory cells, leading to increased proliferation, including that of macrophages. As the number of macrophages increases, the expression of TGF-β by these macrophages also increases. Consequently, collagen synthesis is enhanced, resulting in faster and more effective wound healing [34]. Numerous studies have demonstrated the effectiveness of PBM in stimulating wound healing. Research conducted by Gupta et al. (2015) involved wound healing therapy in mice using PBM with a wavelength of 904 nm, which resulted in accelerated healing, reduced inflammation (histologically), decreased expression of TNFα and NF-kB, and increased expression of VEGF, FGFR-1, HSP-60, HSP-90, HIF-1α, and matrix metalloproteinases-2 and 9 compared to the control group [35]. In the research conducted by Astuti et al. (2021), a red laser light source with a wavelength and energy of 3,332 ± 0.01 mW was applied to experimental Wistar rats with wounds resulting from the removal of their first molar teeth. The study aimed to examine the effect of this laser therapy on the wound-healing process. The parameters observed included lymphocyte cells, fibroblast cells, the formation of new blood vessels, Interleukin 1β (IL-1β), and collagen 1α (COL-1α). These observations were compared with two groups of mice that received either 0.1% antiseptic treatment or antibiotic therapy. The results of the observations indicate that red laser photobiomodulation can aid in wound recovery following tooth extraction. This is evidenced by an increase in the number of lymphocyte cells and fibroblast cells, the formation of new blood vessels, the expression of COL-1α, and a decrease in the expression of IL-1β [2].

PBM was discovered almost 50 years ago by Endre Mester in Hungary [37]. PBM is often referred to as low-level laser therapy because the initial devices used were ruby (694 nm) and HeNe (633 nm). Various wavelengths in the red spectrum (600-700 nm) and near-infrared (NIR, 770-1200 nm) range have demonstrated positive results. More recently, blue and green wavelengths have also been explored, but they encounter significant challenges with penetration depth. Therefore, the wavelength highly recommended for reducing pain or wound healing therapy falls within the red spectral range (600-700 nm). It is widely accepted that light penetration into tissue is influenced by its absorption and scattering by molecules and structures within the tissue. Absorption and scattering decrease considerably as wavelength increases, with the maximum NIR penetration depth being approximately 810 nm [18].

Wound healing therapy continues to be a topic of ongoing development in the present day. Correct, effective, and efficient wound management is of utmost importance. The post-extraction wound healing process typically takes a significant amount of time, often up to 16 weeks. The extended duration of the natural wound healing process is primarily due to various disturbances that can occur. These disturbances can be classified into local and systemic factors [38]. Local factors encompass issues such as infection, compromised blood flow, foreign objects that can interfere with the inflammatory mediator response, mobility, and the wound's size, type, and location. On the other hand, systemic factors include considerations like age, nutritional status, the use of glucocorticoids, uncontrolled diabetes, and haematological abnormalities. Considerable efforts have been dedicated to wound care, focusing on the development of novel therapeutic approaches and the application of new technologies for the treatment of both acute and chronic wounds. The wound-healing process following tooth extraction requires a significant amount of time. Therefore, this study aims to investigate the impact of diode laser exposure in post-operative cases of tooth extraction.

Materials and Methods

In this study, there were 60 subjects: 30 in the therapy group and 30 in the control group. The therapy group underwent photobiomodulation therapy using a 650 nm red laser for 60 seconds, with a radiation dose of 2 J/cm^2. This therapy directed perpendicular light onto the injured area immediately after the extraction procedure. Photobiomodulation therapy using a 650 nm red laser was administered on days 1, 3, and 5 following the extraction procedure. Saliva samples were collected from the subjects, with approximately 15 ml of saliva collected from each individual. In contrast, the control group did not receive photobiomodulation therapy.

Laser source

The laser diode light source used in the study is a red laser with a wavelength of 650 nm. Characterization was conducted using a Jasco CT-10 monochromator to determine the peak wavelength. The power output was measured at 3.33 ± 0.01 mW using an OMM-6810B-220 V power meter. The measurement distances ranged from 1 cm to 5 cm, with intervals of 0.5 cm. Diode laser irradiation was performed with exposure times ranging from 300 to 500 seconds, with 1-second intervals. I was characterizing laser exposure temperature involved directing a laser beam at a thermometer sensor and recording the temperature for 1 second for 300 seconds. The irradiation time is determined as follows to calculate the energy density value [25].

$$\text{Energy Density (J.cm}^{-2}\text{)} = \text{Intensity (W.cm}^{-2}\text{)} \times \\ \times \text{Irradiation Time (s)}. \quad (1)$$

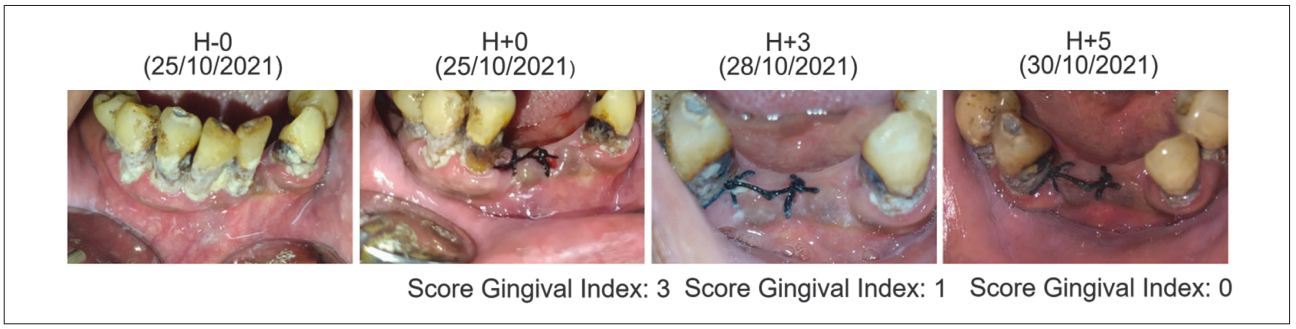

Рис. 1. Оценка десневого индекса.
Fig. 1. Gingival index score.

Gingival Index (GI) Measurement

Samples were collected from gingival index examinations conducted on subjects at the Surgery and Oral Department of RSGM. The baseline data included dental index examinations and the initial saliva collection. The first evaluation occurred after three days of providing laser therapy for gingivitis. The second evaluation was conducted on day five following laser therapy. Gingivitis was assessed using the Gingival Index (GI) developed by Loe and Silness during each evaluation. The Gingival Index scores and corresponding criteria used to determine the gingival status were as follows: 0 (normal gingiva), 1 (mild inflammation characterized by slight changes in colour and mild oedema without bleeding on probing), 2 (moderate inflammation with redness, oedema, and shininess, accompanied by bleeding upon probing), and 3 (severe inflammation marked by pronounced redness and oedema, ulceration with a tendency for spontaneous bleeding) (Anggraini et al., 2016). All scores were recorded, including the gingival index scores of the samples collected before laser therapy (on day 1, day 3, and day 5). The Gingival Index is depicted in Fig. 1.

Enymed-Linked Immunosorbent assay (ELISA)

Enzyme-Linked Immunosorbent Assay (ELISA) was performed using Interleukin 1β (IL-1β), Prostaglandin E2 (PGE2), and Human Beta Defensin 2 (HBD2). ELISA is a biochemical technique employed to detect the presence of antibodies or antigens in a sample. ELISA can test various types of antigens, haptens, or antibodies. The fundamental principle behind the ELISA technique relies on a specific interaction between antibodies and antigens, with enzymes serving as markers. These enzymes produce a signal indicating an antigen's presence if it has reacted with the antibodies. This reaction necessitates using specific antibodies that bind to the antigen (Baker et al., 2007). The ELISA technique is predicated on a specific antigen-antibody reaction with high sensitivity and specificity, utilizing enzymes as indicators. The fundamental principle of ELISA involves analyzing the interaction between antigens and antibodies, with enzymes serving as reaction markers (Yusrini, 2005). ELISA's working principle entails forming a complex between the antigen and antibody, followed by adding specific substrates and peroxidase enzymes. This combination results in a colour change in positive results. ELISA data is typically represented using optical density (OD) values and logarithmic concentrations to generate a sigmoidal curve. This curve can be constructed by direct graphing or by utilizing curve-fitting software in an ELISA reader, such as MS Excel.

Statistical Analysis

In this research, statistical tests were conducted using IBM SPSS to determine if there were significant differences in PGE2, IL-1β, HBD2, and Gingival Index data results. The Independent-Sample T-test was utilized to assess the levels of PGE2, IL-1β, and HBD2, while the Mann-Whitney U test was employed to evaluate the gingival index value.

Results

Interleukin-1β (IL-1β) protein testing was conducted to monitor inflammation during the wound healing process, both before extraction (day 0) and after extraction (day three and day five post-wound occurrence). In general, the observation results indicated a decrease in the levels of IL-1β. The normality test,

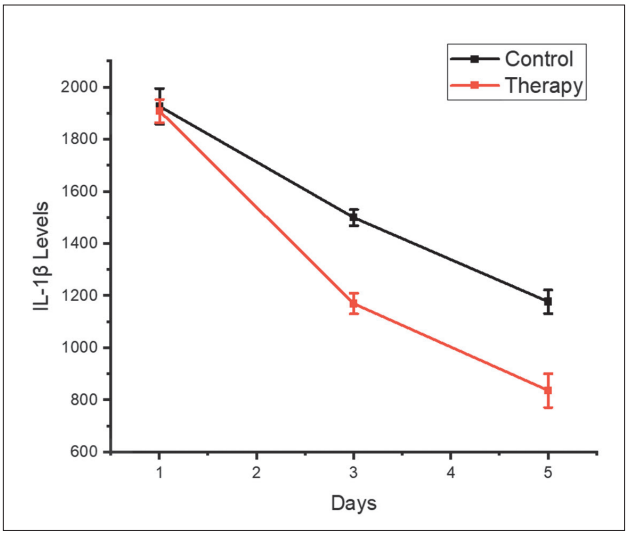

Рис. 2. Динамика уровня интерлейкина-1β в контрольной и терапевтической группах на 1-5 дни.
Fig. 2. Dynamics of Interleukin-1β levels in the control and therapy groups on days 1 to 5.

performed using Kolmogorov-Smirnov, demonstrated that the data exhibited a normal distribution for the control group (without photobiomodulation therapy) with a significance level (α) of 0.300, while for the photobiomodulation therapy group, it was 0.115. Table 1 shows that post-extraction on day 1, day 3, and day five significantly impacted IL-1β levels ($p < 0.05$). All control group subjects exhibited higher IL-1β levels when compared to the photobiomodulation therapy group.

Based on Fig. 2, it is evident that the treatment group receiving 650 nm wavelength red laser photobiomodulation therapy exhibited lower levels of interleukin-1β on day 1, day 3, and day five compared to the control group.

Prostaglandin E2 (PGE2) protein testing was conducted to assess inflammation during the wound healing process on days 1 (pre-extraction) and post-extraction on days three and 5. Overall, the observation results indicate a decrease in the levels of PGE2. The results

Таблица 1
Динамика уровней интерлейкина-β в контрольной и терапевтической группах на 1-й, 3-й и 5-й дни
Table 1
Dynamics of interleukin-1β levels in the control and therapeutic groups on days 1, 3 and 5

Дни Days	Группа Group	N	Среднее Average	Стандартное отклонение SD	Результаты независимого Т-теста Independent T Test Results	
					Результаты Results	Заключение Conclusion
1	Контрольная Control	30	1927.53	68.40	t=7.22	Статистически достоверная разница There are different meanings
	Терапевтическая Therapy	30	1822.20	41.38	p=0.00	
3	Контрольная Control	30	1500.13	30.57	t=41.99	Статистически достоверная разница There are different meanings
	Терапевтическая Therapy	30	1132.10	37.01	p=0.00	
5	Контрольная Control	30	1177.40	45.35	t=25.85	Статистически достоверная разница There are different meanings
	Терапевтическая Therapy	30	820.73	60.44	p=0.00	

Таблица 2
Динамика уровней PGE2 в контрольной и терапевтической группах на 1-й, 3-й и 5-й дни
Table 2
Dynamics of PGE2 levels in the control and therapeutic groups on days 1, 3 and 5

Дни Days	Группа Group	N	Среднее Average	Стандартное отклонение SD	Результаты независимого Т-теста Independent T Test Results	
					Результаты Results	Заключение Conclusion
1	Контрольная Control	30	565.80	10.31	t=5.06	Статистически достоверная разница There are different meanings
	Терапевтическая Therapy	30	554.30	6.96	p=0.00	
2	Контрольная Control	30	493.59	38.55	t=5.48	Статистически достоверная разница There are different meanings
	Терапевтическая Therapy	30	447.23	21.93	p=0.00	
3	Контрольная Control	30	468.07	45.35	t=9.19	Статистически достоверная разница There are different meanings
	Терапевтическая Therapy	30	425.97	20.14	p=0.00	

of the Kolmogorov-Smirnov normality test showed that the data exhibited a normal distribution for the control group (without photobiomodulation therapy) with a significance level (α) of 0.37, while for the therapy group (with photobiomodulation therapy), it was 0.32.

As shown in Table 2, the post-extraction procedures on day 1, day 3, and day 5 had a significant impact on PGE2 levels ($p < 0.05$). The control group subjects had higher PGE2 levels than the photobiomodulation therapy group.

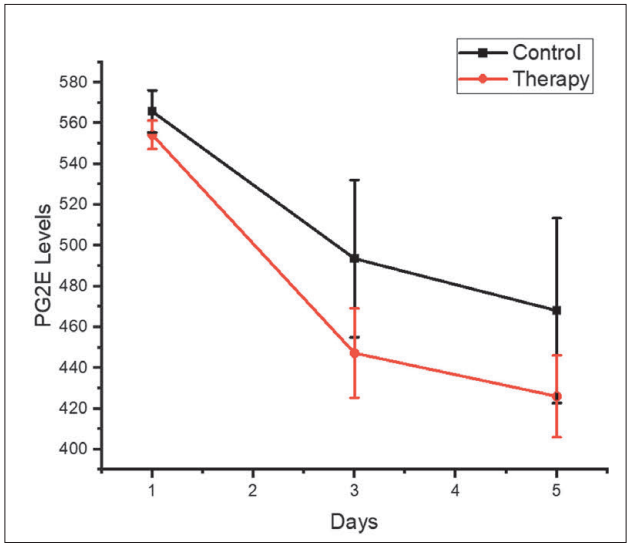

Рис. 3. Уровни PGE2 в контрольной и терапевтической группах в 1-5 дни.
Fig. 3. Dynamics of PGE2 levels in the control and therapy groups on days 1 to 5.

Based on Fig. 3, it is evident that the treatment group receiving 650 nm wavelength red laser photobiomodulation therapy exhibited lower PGE2 levels on day 1, day 3, and day 5 in comparison to the control group.

Human β defensin 2 (HBD2) is a small protein consisting of 15-20 residues that play a role in antimicrobial defence by penetrating microbial cell membranes and inducing microbial death, similar to the action of antibiotics. The observation of the HBD2 protein was conducted to identify indications of inflammation during the wound healing process on day 1 (pre-extraction) and post-extraction on day three and day 5. Overall, the observation results indicate a decrease in the levels of HBD2. The results of the normality test using Kolmogorov-Smirnov revealed that the data exhibited typical distribution characteristics for the control group (without photobiomodulation therapy) with a significance level (α) of 0.186 and for the therapy group (with photobiomodulation therapy), it was 0.171.

Table 3 shows that the post-extraction procedures on day 1, day 3, and day five significantly impacted the levels of HBD2 ($p < 0.05$). The control group subjects exhibited higher HBD2 levels when compared to the photobiomodulation therapy group.

Based on Fig. 4, it is apparent that the treatment group receiving 650 nm wavelength red laser photobiomodulation therapy displayed lower HBD2 levels on day 1, day 3, and day five compared to the control group.

The gingival index (GI) is employed to evaluate gum inflammation severity. It involves measuring six selected teeth, serving as index teeth, including the upper right

Таблица 3
Динамика уровней HBD2 в контрольной и терапевтической группах на 1-й, 3-й и 5-й дни
Table 3
Dynamics of HBD2 levels in the control and therapeutic groups on days 1, 3 and 5

Дни Days	Группа Group	N	Среднее Average	Стандартное отклонение SD	Результаты независимого Т-теста Independent T Test Results	
					Результаты Results	Заключение Conclusion
1	Контрольная Control	30	1864.77	10.31	t=2.29	Статистически достоверная разница There are different meanings
	Терапевтическая Therapy	30	1843.60	6.96	p=0.00	
2	Контрольная Control	30	1817.27	38.55	t=7.22	Статистически достоверная разница There are different meanings
	Терапевтическая Therapy	30	1666.90	21.93	p=0.00	
3	Контрольная Control	30	1659.57	45.35	t=8.28	Статистически достоверная разница There are different meanings
	Терапевтическая Therapy	30	1517.37	20.14	p=0.00	

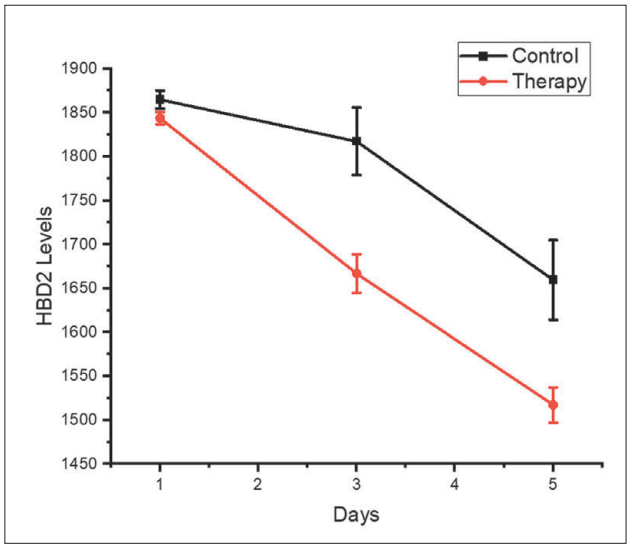

Рис. 4. Динамика уровня HBD2 в контрольной и терапевтической группах с 1 по 5 день.
Fig. 4. Dynamics of HBD2 levels in the control and therapy groups on day 1 to day 5.

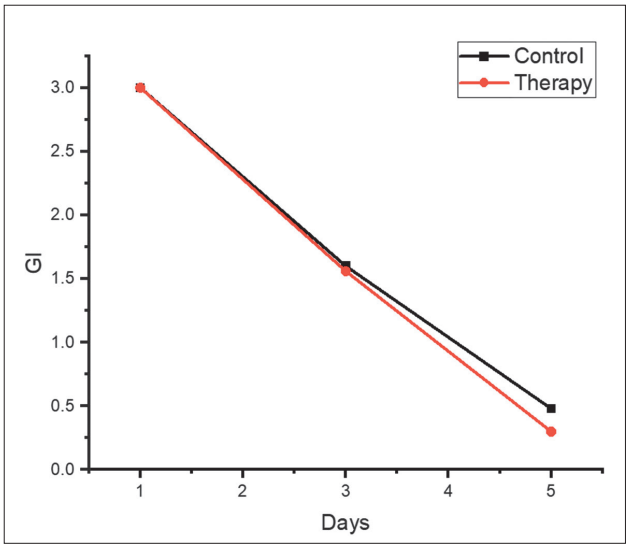

Рис. 5. Динамика десневого индекса в терапевтической группе и контрольной группе с 1 по 5 день.
Fig. 5. Dynamics of GI in the therapy group and control group on day 1 to day 5.

first molar, upper left first incisor, upper left first premolar, lower left first molar, lower right first incisor, and lower right first premolar. GI assesses gum inflammation for each tooth in various aspects (facial, mesial, distal, lingual), assigning a score ranging from 0 to 3 for both the control and therapy groups. A Non-Parametric Test is performed since the data takes the form of interval data.

Based on the outcomes of the non-parametric T-Test for two independent samples utilizing the Mann-Whitney U method, a p-value of 0.32 was obtained on day 1, indicating no significant difference between the control group and the therapy group. However, on day three and day 5, a p-value of less than 0.05 was obtained, signifying a significant difference between the control and therapy groups.

Based on Fig. 4, it is evident that on day 0 and day 1, the GI values were identical, indicating no difference between the control and therapy groups. However, on day three and day 5, differences in GI values were observed. These days, the GI value in the therapy group was lower than the GI value in the control group.

Таблица 4
Динамика десневого индекса в контрольной и терапевтической группах на 1-й, 3-й и 5-й дни
Table 4
Dynamics of Gingival Index levels in the control and therapeutic groups on days 1, 3 and 5

Дни Days	Группа Group	N	Среднее Average	Результаты теста Манна-Уитни Mann-Whitney U Test Results	
				Результаты Results	Заключение Conclusion
1	Контрольная Control	30	3.00	p=0.32	Нет статистически достоверной разницы There is no difference in meaning
	Терапевтическая Therapy	30	3.00		
3	Контрольная Control	30	1.60	p=0.01	Статистически достоверная разница There are different meanings
	Терапевтическая Therapy	30	1.56		
5	Контрольная Control	30	0.48	p=0.03	Статистически достоверная разница There are different meanings
	Терапевтическая Therapy	30	0.30		

Discussion

Tooth extraction is performed in response to issues arising in the oral cavity, such as bacteria, disease, or trauma, that render tooth retention untenable. Following tooth extraction, tissue around the extracted tooth sustains injury. The wound-healing process is the human body's defensive reaction to various injuries. This intricate and dynamic process encompasses inflammation, proliferation, and remodelling phases, each supported by mediators playing specific roles. In response to the wound, the body carries out the physiological function of wound healing. The healing process encompasses three phases: the initial, intermediate, and advanced, each characterized by unique biological processes and cell functions. In the initial phase, hemostasis occurs, during which blood vessels severed in the wound undergo vasoconstriction to halt blood flow. This phase initiates inflammation, clears damaged tissue, and prevents bacterial infection. Chemical agents can assist in the wound-healing process. Low-level laser therapy has long been recognized for stimulating cell activity, including inflammatory cells, which play a pivotal role in wound healing [39].

Photobiomodulation is a device that utilizes coherent, collimated, and monochromatic light energy. It is a light therapy source that significantly relies on the wavelength and energy it employs [35]. Photobiomodulation operates with low wavelengths, energy levels, and doses, enabling it to deliver therapeutic effects to illuminated tissues. Characterization conducted in this research demonstrated that red laser light, with a wavelength of 650 nm, had a measured wavelength and energy of 3.332 ± 0.01 mW. Photon energy is beneficial in augmenting kinetic energy and activating or deactivating enzymes. The response of cells and tissues to growth factors can be observed through various indicators, such as increased ATP and protein synthesis, alterations in cell membrane permeability, calcium ion absorption, cell proliferation, and a series of metabolic changes that ultimately lead to physiological modifications facilitating tissue repair. The favourable clinical outcomes of photobiomodulation (PBM) include anti-inflammatory effects, analgesic properties, pain suppression, and enhanced healing in irradiated tissues. Achieving a specific power density and precise irradiation levels are crucial factors in the interaction between lasers and tissue. Peplow et al. (2010) research emphasized the necessity of using low power levels in photobiomodulation therapy, with dosage playing a pivotal role in its effectiveness [40].

Laser therapy relies on low-intensity lasers or LED lights, mainly through photobiomodulation (PBM) techniques. One of the most intriguing aspects of PBM is its impact on stem cells and progenitor cells, which can lead to increased differentiation rates and ultimately accelerate tissue healing [41]. Numerous studies have indicated that PBM can enhance stem cell proliferation, including gingival fibroblasts and dental pulp stem cells obtained from exfoliated permanent and primary teeth. The effectiveness of PBM on target tissues is contingent on various parameters, including the light source, wavelength, energy density, and duration of laser exposure. Photobiomodulation (PBM) is a non-invasive therapy that effectively reduces inflammation and alleviates pain. It involves the therapeutic use of coherent, collimated, monochromatic, and polarized light absorbed by an endogenous chromophore called cytochrome C. This absorption triggers non-thermal and non-cytotoxic biological reactions through photochemical and photophysical events, ultimately leading to physiological changes.

Low-density energy and specific wavelengths used in PBM therapy facilitate light penetration into cells and tissues, resulting in modulatory effects. These effects include the modulation of inflammation, the proliferation of endothelial cells stimulated by growth factors like VEGF, and increased fibroblast proliferation, which, in turn, enhances collagen synthesis. These events are considered crucial for the proper progression of the healing process. The efficiency of PBM in influencing cellular mechanisms, whether related to proliferation, energy pathways, electrical signal transduction, biochemical processes, or immune activity, is directly dependent on the specific parameters used. These parameters include electromagnetic wavelength, dose, light beam area, tissue specificity, time of exposure, and the type of injury being treated [22].

Interleukin-1 beta (IL-1β) is a pro-inflammatory cytokine that plays a role in various physiological processes, including pain, inflammation, and autoimmune conditions. It is primarily produced by activated macrophages. IL-1β has several functions, such as stimulating thymocyte proliferation by inducing the release of interleukin-2 (IL-2), promoting B cell maturation and proliferation, and enhancing fibroblast growth factor activity. Additionally, IL-1β is involved in the inflammatory response, acts as an endogenous pyrogen, and can stimulate the release of prostaglandins and collagenase from synovial cells.

During inflammation, IL-1β levels typically show a significant increase, unlike IL-1α. Type 1 interleukin tends to increase and decrease during the inflammatory process's proliferation phase. In some instances, a significant increase in IL-1β and tumour necrosis factor-alpha (TNF-α) levels may be observed in non-improving foot wounds, indicating ongoing inflammation. The release of IL-1β as a pro-inflammatory cytokine is a natural response during the inflammatory phase of the wound healing process. It is crucial in the body's defence against microorganisms and pathogens. In normal tissue conditions, the expression of IL-1β is generally low. However, during inflammation, the release of IL-1β

increases significantly as it contributes to the immune response and the destruction of pathogens in the affected area. This study has shown differences in the levels of IL-1β protein during the wound healing process after tooth extraction when red laser photobiomodulation therapy at a wavelength of 650 nm was applied.

Fig. 2 indicates that the reduction in IL-1β levels was more pronounced in the therapy group compared to the control group. This suggests that photobiomodulation therapy with red laser light reduced this pro-inflammatory cytokine's levels during the healing process. Furthermore, photobiomodulation has also been associated with reduced levels of inflammatory cytokines in nerve injuries, leading to pain reduction and facilitating nerve regeneration, as evidenced by changes in TNF-α, IL-1β, and GAP-43 levels [42].

Prostaglandins play a significant role as inflammatory mediators, and inhibiting prostaglandin production can reduce inflammation. In this study, Prostaglandin E2 (PGE2) levels, which indicate inflammation during the wound healing process, were assessed using enzyme-linked immunosorbent tests (ELISA). The observations revealed a general decrease in the levels of PGE2.

The results of the ELISA tests on saliva samples demonstrated a significant difference between the control and therapy groups. The Independent-Sample T-Test results indicated that on day 1, day 3, and day 5, the red laser photobiomodulation therapy group showed $\alpha<0.05$, signifying a significant difference between the two groups.

This finding is consistent with research conducted by Lim et al. (2015), who investigated the effects of lipopolysaccharide (LPS) from Porphyromonas gingivalis on human gingival fibroblasts (HGF). The study suggests that red laser photobiomodulation therapy has the potential to modulate PGE2 levels, contributing to the reduction of inflammation during the wound healing process [43]. The study by Lim et al. (2015) used Photobiomodulation with a wavelength of 650 nm and applied it to cells exposed to lipopolysaccharide (LPS) either directly or indirectly (by transferring media from PBM-treated cells to other cells with LPS). Both direct and indirect protocols resulted in reductions in various inflammatory markers, including cyclooxygenase-2 (COX2), prostaglandin E2 (PGE2), granulocyte colony-stimulating factor (GCSF), regulated on activated normal T cells expressed and secreted (RANTES), and CXCL11. These findings suggest that Photobiomodulation with a 650 nm wavelength has the potential to effectively reduce inflammation, as indicated by the decreased levels of these inflammatory markers.

The ELISA test results for Human β defensin 2 (HBD2) parameters indicate a difference in the decrease in HBD2 levels between the control and therapy groups. The Independent Sample T-Test results on days 1, 3, and 5 showed a significant difference between the two groups, with $p<0.05$ in each case. This means the therapy group exhibited a more significant reduction in HBD2 levels than the control group. These findings suggest that the red laser photobiomodulation therapy at a wavelength of 650 nm had a notable impact on reducing HBD2 levels, which may contribute to its beneficial effects on wound healing.

In this study, gingival inflammation occurs due to toxins released by bacteria, leading to irritation, redness, and swelling of the gingiva. The degree of gingival inflammation can be assessed using the Gingival Index [46]. The research findings indicated differences in Gingival Index values during wound healing when utilizing red laser photobiomodulation therapy at 650nm. The Mann-Whitney U statistical analysis revealed no significant difference between the control and therapy groups on day 1 ($p=0.32$). However, on the third day, a significant difference was observed with a p-value of 0.01, and on day 5, a significant difference was also noted with a p-value of 0.03. These results suggest that the photobiomodulation therapy group had a lower Gingival Index value than the control group, indicating a positive impact on reducing gingival inflammation.

Conclusion

Based on the research results, it is evident that there is a decrease in the values of IL-1β, PGE2, HBD2, and GI. This indicates that local immune cells, including resident macrophages, are activated by proinflammatory mediators released in response to injury, significantly accelerating the wound-healing process.

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ORIGINAL ARTICLES

EFFECTS OF SILVER NANOPARTICLE AND LOW-LEVEL LASER ON THE IMMUNE RESPONSE AND HEALING OF ALBINO MICE SKIN WOUNDS

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Abstract

The structural integrity of the skin, which acts as a barrier to keep harmful external substances out of the body, is compromised by wounds. The process of wound healing is a multifaceted and ever-changing phenomenon that entails the replacement of bodily tissues or damaged skin. It has been demonstrated that nanoparticles, especially silver nanoparticles, have anti-microbial and anti-inflammatory qualities that encourage cell migration and proliferation. Low level laser therapy has the potential to accelerate wound healing by stimulating cell regeneration after injury, reducing pain, and modulating the immune system. The aim of our study is to evaluate the healing process after treatment with silver nanoparticle and/or low level laser by measuring the serum levels of some pro-inflammatory cytokines (IL1b, IL6, and TNF-α), wound healing rate and histological analysis. Wounds were inflicted into 63 adult male albino mice (Mus musculus) and randomly divided into nine groups (7 per each). Control was left to normal healing. Other groups received a different treatment with laser, silver nanoparticle or both for 21 days. Injured skin was sampled for histopathological examination. Quantitative determination of TNFα, IL1 beta and IL6 were carried out using the sandwich enzyme-linked immunosorbent assay (ELISA) twice (day 2 and day 21). One-way and two-way analysis of variance (ANOVA) was used for statistical analysis. The results showed that the groups treated with silver nanoparticles and / or low-level laser promoted wound healing by reducing pro-inflammatory cytokines (IL1β, IL6 and TNFα) and showed significantly better wound closure with a significant reduction in wound size. At day 2 histopathological changes were very similar in different groups. When silver nanoparticles were applied, either alone or in combination with laser exposure, better granulation tissue and fibrosis also necrosis in the canter of the lesion and high score of re-epithelialization with less inflammation observed gradually till day 21. The results of this study suggested that silver nanoparticles and low-level laser have a wound healing potential, since topical treatment with silver nanoparticles and low-level leasers has effectively improved the wound healing process.

Key words: wound healing, nanoparticle, silver nanoparticle, low level laser (LLL), pro-inflammatory cytokines, AgNPs, IL6, IL1β, TNF- α.

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ВЛИЯНИЕ НАНОЧАСТИЦ СЕРЕБРА И НИЗКОИНТЕНСИВНОГО ЛАЗЕРА НА ИММУННЫЙ ОТВЕТ И ЗАЖИВЛЕНИЕ КОЖНЫХ РАН МЫШЕЙ-АЛЬБИНОСОВ

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Резюме

Структурная целостность кожи, которая действует как барьер, препятствующий проникновению вредных внешних веществ в организм, нарушается ранами. Процесс заживления ран влечет за собой замену тканей организма или поврежденной кожи. Было продемонстрировано, что наночастицы, особенно наночастицы серебра, обладают антимикробными и противовоспалительными свойствами и стимулируют миграцию и пролиферацию клеток. Низкоинтенсивная лазерная терапия может ускорить заживление ран за счет стимуляции регенерации клеток после травмы, уменьшения боли и модуляции иммунной системы. Целью нашего исследования является оценка процесса заживления после лечения наночастицами серебра и/или низкоинтенсивным лазером путем измерения сывороточных уровней некоторых провоспалительных цитокинов (IL1b, IL6 и TNF-α), скорости заживления ран и гистологического анализа. Раны были нанесены 63 взрослым самцам мышей-альбиносов (Mus musculus). Мыши были случайным образом разделены на девять групп по 7 мышей. Контрольная группы была оставлена без воздействия до нормального заживления. Другие группы получали другое лечение лазером, наночастицами серебра или и тем, и другим в течение 21 сут. Поврежденная кожа была взята для гистопатологического исследования. Количественное определение TNFα, IL1 бета и IL6 проводили с помощью иммуноферментного анализа (ИФА) дважды (2 и 21 сут). Для статистического анализа применяли однофакторный и двуфакторный дисперсионный анализ

(ANOVA). Результаты показали, что в группах, получавших воздействие наночастицами серебра и/или низкоинтенсивным лазером, заживление ран сопровождалось увеличением уровней провоспалительных цитокинов (IL1β, IL6 и TNFα). В этих группах было показано сокращение времени закрытия раны со значительным уменьшением размера раны. На 2-й день гистопатологические изменения были очень похожи в разных группах. При нанесении наночастиц серебра, отдельно или в сочетании с лазерным воздействием, наблюдалось ускоренное образование грануляционной ткани и фиброза, а также некроз в области поражения. В тих группах был получен более высокий балл реэпителизации с меньшим воспалением (до 21 сут). Результаты данного исследования свидетельствуют о том, что наночастицы серебра и низкоинтенсивный лазер обладают ранозаживляющим потенциалом, так как местное применение наночастицам серебра и низкоинтенсивного препаратами эффективно улучшило процесс заживления ран.

Ключевые слова: заживление раны, наночастицы, наночастицы серебра, низкоинтенсивный лазер, провоспалительные цитокины, AgNPs, IL6, IL1β, TNF-α.

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Introduction

Every year, millions of surgical incisions are caused during standard medical care [1]. One of the fundamental goals of clinical care continues to be promoting the healing of these inadvertent and intentional injuries, reducing the cosmetic impact on the patient, and maximizing the restoration of tissue function with the least number of scars [2]. When an injury occurs, damaged wound tissue is naturally repaired by a series of intricate cellular and biomolecular processes that bring it back to its pre-injury state [3]. The inflammatory response and related cellular migration, proliferation, matrix deposition, and tissue remodeling make up the fundamental biological mechanism of wound healing. When the healing processes proceed in a well-organized manner, the wound heals quickly and, in the case of acute wound healing, leaves little to no visible scars [4].

In healthy individuals, this mechanism functions at its best; nevertheless, there are many reasons that lead to poor wound healing, including aging, trauma, surgery, and acute or chronic illnesses like diabetes mellitus (DM) [5,6]. According to DeClue and Shornick's [7] research, the overproduction of pro-inflammatory cytokines including TNF-α, IL1β, and IL6 is linked to poor wound healing.

One of the key components of wound healing is thought to be the inflammatory response. The different inflammatory mediators are released to control the wound-healing process. Pro- and anti-inflammatory cytokines may be produced during normal wound healing, and the inflammatory response is more than sufficient. Encouraging wound healing and tissue regeneration requires safe in vivo regulation of the inflammatory response [8]. Deregulation of proinflammatory cytokines, including TNFα and IL1β, prolongs the inflammatory phase and slows healing [8,9].

Interleukin (IL)-1α/IL1β have both been promoted as "master regulators" of the wound healing response due to the large number of processes each regulates after injury or infection [10]. IL1β is released primarily by monocytes and macrophages as well as by nonimmune cells, such as fibroblasts and endothelial cells, during cell injury, infection, invasion, and inflammation [11].

IL6 is produced by neutrophils and monocytes and has been shown to be important in initiating the healing response. Its expression is increased after wounding and tends to persist in older wounds [12].

IL6 levels in wound fluids correlated with wound-healing rates [13]. TNF-α, at low levels, can promote wound healing by indirectly stimulating inflammation and increasing macrophage produced growth factors. However, at higher levels, especially for prolonged periods of time, TNF-a has a detrimental effect on healing [13,14].

NPs are tiny particles having a size range of 1–100 nm. They have unique properties such as size, shape, large surface area to volume ratio etc. NPs due to their vast range of antimicrobial property and rapid effectiveness with minimal dose are one of the choices of researchers for wound healing [15]. Conventional wound healing drugs have limited potential as they cannot penetrate the cell membrane, which a NP can [16]. Regulatory approval of Nano pharmaceuticals is slow, as the Food and Drug Administration (FDA) should approve them [16,17].

Silver is a widely used as antimicrobial agent in health care products. It has been applied for centuries in sanitization, health care, and to inhibit bacteria in food, but it has only been introduced into wound care as an antibacterial in recent years [18]. The use of silver in the past has been restrained by the need to produce silver as a compound; thereby increasing the potential effects. Nanotechnology has provided a way of producing pure silver nanoparticles [19]. This system also markedly increases the rate of silver ion release.

Laser light has the unique properties of monochromaticity (single wavelength), collimation (runs in one direction without divergence), and coherence

(with all waves in phase). These properties allow laser light to penetrate the skin's surface non-invasively [20]. Therapeutic lasers are a thermic without significant heat transfer (<0.65°C); in this way, photon energy is transferred directly to the target cells and thermal damage is avoided. Therapeutic lasers use monochromatic light in the 630 to 905 nm range, also known as the «therapeutic window» [21]. Laser therapy could enhance wound healing process by stimulating cell regeneration after injury, attenuating pain, and modulating the immune system [22].

The aim of our study is to evaluate the healing process after treatment with silver nanoparticle and/or low-level laser by measuring the serum levels of some Pro-inflammatory cytokines (IL1 β, IL6, and TNF-α), wound healing rate and histological analysis.

Materials and Methods

Silver nanoparticles

20% nano colloidal silver nanoparticles were purchased from (Paradise HealthCare)Spherical morphology with particle size ranged less than 10 nm (i.e. Aggregates as 200 atoms of Silver) [23].

Moisten infected area with colloidal silver (using cotton swabs) daily for the treated groups.

Laser treatment Exposure

Selected groups were exposed to diode laser (LSR_PS_ II # 10042504- Germany) light three times a week, for up to 21 days, with a radiation power of 650 ± 5 nm; emits 180 mW/ cm [2,24], with a 6 ml spot size at 20 cm from the injured part of the animal for 5 minutes.

Animals

In this study, 63 healthy adult male albino Swiss mice (Mus musculus) weighing 25 ± 5 g were purchased from the National Research Center (Dokki, Egypt), kept in standard polypropylene cages and acclimatized one week before the experimental work. The animals were housed at the animal house Zoology Department, Faculty of Science, Cairo University and had free access to standard pellet feed throughout, according to the test protocol, and had free access to water for the entire duration of the test. The animals were kept under standard conditions: temperature (25 ± 2ºC), relative humidity of the environment (55%) and alternating light-dark cycle (12 h / 12 h).

Animals were divided into nine groups as follows:

Group N: baseline, normal, without injury, three mice were euthanized at day3 and the others were euthanized at day 21; **Group W day 2:** positive control, injured, untreated (inflammatory phase) and were euthanized at day 2; **Group W day 21:** wounded, untreated, (remodeling phase) and were euthanized at day 21; **Group W + L day 2**: wounded treated once with laser light and were euthanized at day 2; G**roup W + L day 21**: wounded treated with laser light 3 times a week for 21 days and then were euthanized; **Group W + Silver day 2:** wound treated once with silver nanoparticle and were euthanized at day 2; **Group W + Silver day 21:** wounded treated with silver nanoparticle three times a week for 21 days and then were euthanized; **Group W + L + Silver day 2:** wound treated once with laser, followed by silver nanoparticle and then were euthanized at day 2; **Group W + L + Silver day 21:** after each laser session, the wounded were treated with silver nanoparticle three times a week for 21 days and then were euthanized.

Study Design Procedure

Wounds were inflicted on mice. Subsequently, the relevant groups were treated topically with silver nanoparticles and / or low-level laser therapy for the duration of the study (21 days). Positive control was left to normal healing. Groups designed to allow evaluating the selected parameter of the study at the inflammation period (day 2) and the remodeling period (day 21). So, wounded tissue and blood samples were collected at day 2 and 21 days after wounding, study evaluations such as healing rate, histopathological examination, cytokine assay by enzyme-linked immunosorbent assays (ELISA) were performed to confirm the effectiveness of wound healing after treatments.

Induction of wound

Before injury, each animal was partially anesthetized for a few seconds, to facilitate wounding, with an anesthetic solution of isoflurane (Anahal-Pharco-Egypt), the dorsal fur of the animals was shaved with an electric hair clipper and disinfected with 70% ethanol. A standardized full thickness open excisional wound was made using a 6 mm diameter, 2 mm deep biopsy punch (Royaltek, USA) [25,26]. Wound areas were examined on 4th, 7th, 11th ,15th,18th, and 21st day after the injury and evaluated for the percentage of wound contraction as the following equation [27]:

$$\text{Percentage of wound contraction} = \frac{\text{Wound area day 1} - \text{Wound area day } n}{\text{Wound area day 1}} \times 100$$

Histopathological Procedure

Autopsy pieces of wounded skin were collected for histopathological examination, samples fixed in 10% neutral buffered formalin. Washing within tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56°C in hot air oven for 24 h. Paraplast wax (Royaltek, USA) tissue blocks were prepared for sectioning at 4μ thickness by rotatory microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin and eosin stain (PHARCO) [28], then examination was done through the Olympus-USA BX43 light microscope and photographed using the Cellsens dimensions software linked to Olympus DP27 camera USA.

Blood Sampling and Cytokine Assay

Samples were collected from the bleeding orbital venous plexus after short anesthesia by Isoflurane solution, Once the required blood volume was drawn the serum was stored at -80°C then the animal was sacrificed by cervical dislocation. Quantitative determination of TNF-α, IL1β and IL6 levels was carried out with the sandwich enzyme- linked immunosorbent assay (ELISA) according to the manufacturer's instructions, optical density was measured at 450 nm using on Plate Reader (DAS-Italy), diagnostic kits for IL1β, IL6 and TNFα were purchased from Glory Science Co., Ltd-China.

Statistical analysis

All statistical analysis was executed using Statistical Package of the Social Sciences (IIBM-SPCS, version 26). Two ways analysis of variance (ANOVA) was applied to study the effect of silver nanoparticles and/or LLL on wounds and their interaction on the levels of the studied parameters (inflammatory cytokines IL1β, TNFα, IL6; wounds healing rate; histopathological examination and blood count) at day 2 and day 21. The post comparison test was used to detect the significant difference for the studied parameters among the wounded groups at definite time intervals. Data were represented as mean (M) ± standard error (SE). Significant difference was considered at $p<0.05$.

Ethical Statement

The experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC), Faculty of Sciences, Cairo University (Egypt) (CU/I/F/88/19). All experimental procedures were performed in accordance with international guidelines for the care and use of laboratory animals performed in accordance with the recommendations of the current edition of the Guide for the care and use of laboratory animals, 8th edition, 2011, USA.

Results

A low level of inflammation is necessary for faster wound healing, but its high level is destructive and delays it – this is the main objective of our study and that's how we built our methods and selected analysis to evaluate how much we achieved that.

Sixty-three healthy male adult albino mice; divided into nine groups (7 per each group) all except the normal group being circularly wounded at its dorsal as mentioned previously; one group left to normal healing and the others received a topical treatment with low level laser, silver nanoparticles, or both along the experimental period.

Wound healing monitoring, inflammatory cytokines assay and histological analysis all were considered as parameter to conclude the best effect of our treatments. Groups were designed to evaluate the selected parameters during the inflammation, maturation and remodelling phase.

Silver nanoparticles and LLL promote healing process

The re-epithelialization and closure of wounds were observed and measured regularly for 21 days in order to evaluate the progress of healing rate of all groups until the wounds were closed completely. The healing rate were calculated using the equation mentioned previously [36] (Fig. 1).

Wound closure was significantly improved within the injured mice treated with silver, LLL or both compared with the untreated group (normal healing), in untreated mice the wound closure was more severely affected than in treated mice since the wound closure percentage reached 12.74% at day 4 and increased to 28.5% at day 7 till 90.41% at day 21 after injury. In mice treated with LLL, the wound closure percentage reached 18.4% at day 4 after injury and increased to 40.4% at day 7 reached 94.4% at day21 after injury, group treated with silver only is more better as it reached 20.85 at day 4 increased to 45.6% at day7 and finally 98.8 at the end of experiment (day 21). The combined group gave the better healing percentage value as it reached 25.8% at day 4 increased to 55.2% at day7 then only at day18 completely healed 100%.

Next, statistical analysis by LSD indicated that the treatment with either silver nanoparticles or LLL induced a significant increase ($p < 0.01$; LSD) in wound closure percent at days 7 and 14 while both treatments together induced a significant increase ($p < 0.01$; LSD) at days 4, 7 and 18 after injury. Two way ANOVA revealed that the effect between groups was significant ($p < 0.001$) on wound closure percent throughout the experiment; comparison of the means in different groups at different times with Two way ANOVA Duncan Multiple Range Test (DMRT) showed at Table 1.

Среднее ± стандартная ошибка (SE) на основе анализа ANOVA; количество животных в каждой группе – 7; средние значения в одной и той же строке, использующие один и тот же надстрочный символ (символы), существенно не отличаются ($p < 0,05$) по данным Duncan Multiple Range Test (DMRT).

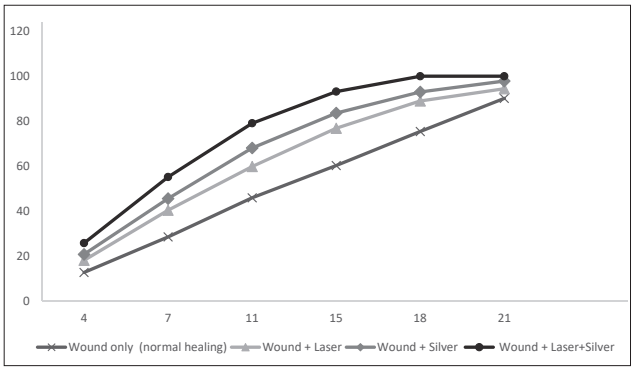

Рис. 1. Динамика уменьшения площади раны (% от исходного) во всех опытных группах в течение 21 дня после нанесения раны.

Fig. 1. The rate of wound contraction (% of initial wound area) in all experimental groups for 21 days post wounding.

Mean ± standard error (SE) based on ANOVA analysis; number of animals in each group is seven; the means in the same row which share the same superscript symbol(s) are not significantly different ($p < 0.05$) according to Duncan Multiple Range Test (DMRT). More over, we compared the appearance of healed wounds by digital photographs. We found that wounds in the Silver and LLL group showed the most resemblance to normal skin, with less hypertrophic scarring and nearly normal hair growth on the wound surface. The worst cosmetic appearance was observed in the normal healing group (Fig. 2). Healed wounds from the treated groups resembled looks as normal skin, with a thin epidermis and normal hair follicles. In contrast, untreated group showed thickened epidermis and no evidence of hair growth.

Histopathology results

Day 2

A surgical wound examination was performed in all experimental groups. Wound healing was very similar in all groups at day 2 after induction. The wound clefts were filled with necrotic tissue, abundant inflammatory cells, mainly neutrophils, edema, and hemorrhage. The wound surface was covered by a serocellular crust. The wound injury score was examined in all groups. No significant difference was found between the different groups with respect to any of the evaluated criteria; re-epithelialization, granulation tissue, inflammation, and angiogenesis (Fig. 3).

Day 21

Variation in wound healing process was found in various test groups after 21 days. The **W Day 21** group showed severe histopathological changes compared to other treated groups. In most individuals, persistence of the necrotic scab without epidermal remodeling was observed, accompanied by transmigration of large numbers of neutrophils. In the wound space, a poor organization of the filler granulation tissue was found, mixed with a strong infiltration of inflammatory cells and a poor angiogenesis process.

In group **L Day 21**, signs of re-epithelialization were found at the edge of the wound, which was characterized by hyperplasia of stratified squamous epithelial cells and partially extended to the wound surface. In the newly formed granulation tissue, less inflammation was found with better angiogenesis process compared to **W Day 21**.

Perfect wound healing was found in the **Silver Day 21** and **L + Silver Day 21** groups. Complete epidermal coverage was observed in most of the sections examined; some individuals in the **L + Silver Day 21** group showed re-epithelialization under the persistent serocellular crust. Organized fibro-vascular tissue occupied the wound clefts of the latter two groups, which were rich in collagen bundles and numerous newly formed blood capillaries.

The wound injury score showed a significant decrease in all parameters in the **W Day 21** group compared to other experimental groups. **L + Silver Day 21** group gave the highest significance observed in all the evaluation

Таблица 1
Анализ скорости заживления обработанных и необработанных ран
Table 1
Treated and non-treated wounds healing rate analysis

Группы Groups	Время наблюдения, сут Time, days					
	4	7	11	15	18	21
Рана Wounded	12.74^{n}	28.55^{K}	45.95^{I}	60.27^{g}	75.42^{e}	90.14^{bc}
Рана + низкоинтенсивный лазер Wounded + Low Level laser	18.14^{m}	40.42^{J}	59.85^{h}	76.85^{f}	89^{c}	94.42^{ab}
Рана + серебро Wounded +Silver	20.85^{m}	45.60^{I}	68.142^{g}	83.65^{d}	93.48^{b}	98.85^{ab}
Рана + низкоинтенсивный лазер + серебро Wounded + Low Level laser +Silver	25.87^{L}	55.12^{I}	79.094^{g}	93.17^{cd}	100.00^{ab}	100.00^{a}

Среднее ± стандартная ошибка (SE) на основе анализа ANOVA; количество животных в каждой группе – 7; средние значения в одной и той же строке, использующие один и тот же надстрочный символ (символы), существенно не отличаются ($p < 0,05$) по данным Duncan Multiple Range Test (DMRT).
Mean ± standard error (SE) based on ANOVA analysis; number of animals in each group is seven; the means in the same row which share the same superscript symbol(s) are not significantly different ($p < 0.05$) according to Duncan Multiple Range Test (DMRT).)

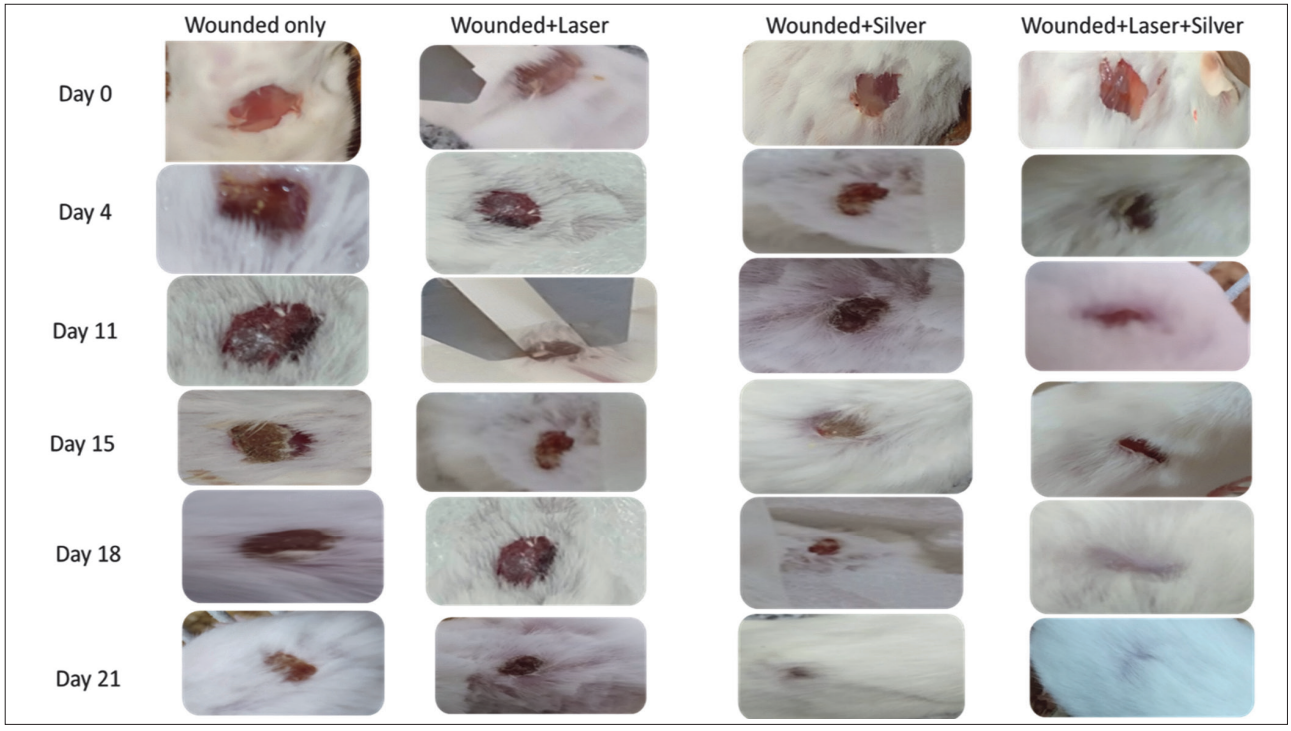

Рис. 2. Цифровые фотографии иссеченных ран всех экспериментальных групп
Fig. 2. Digital photographs of excision wounds of all experimental groups

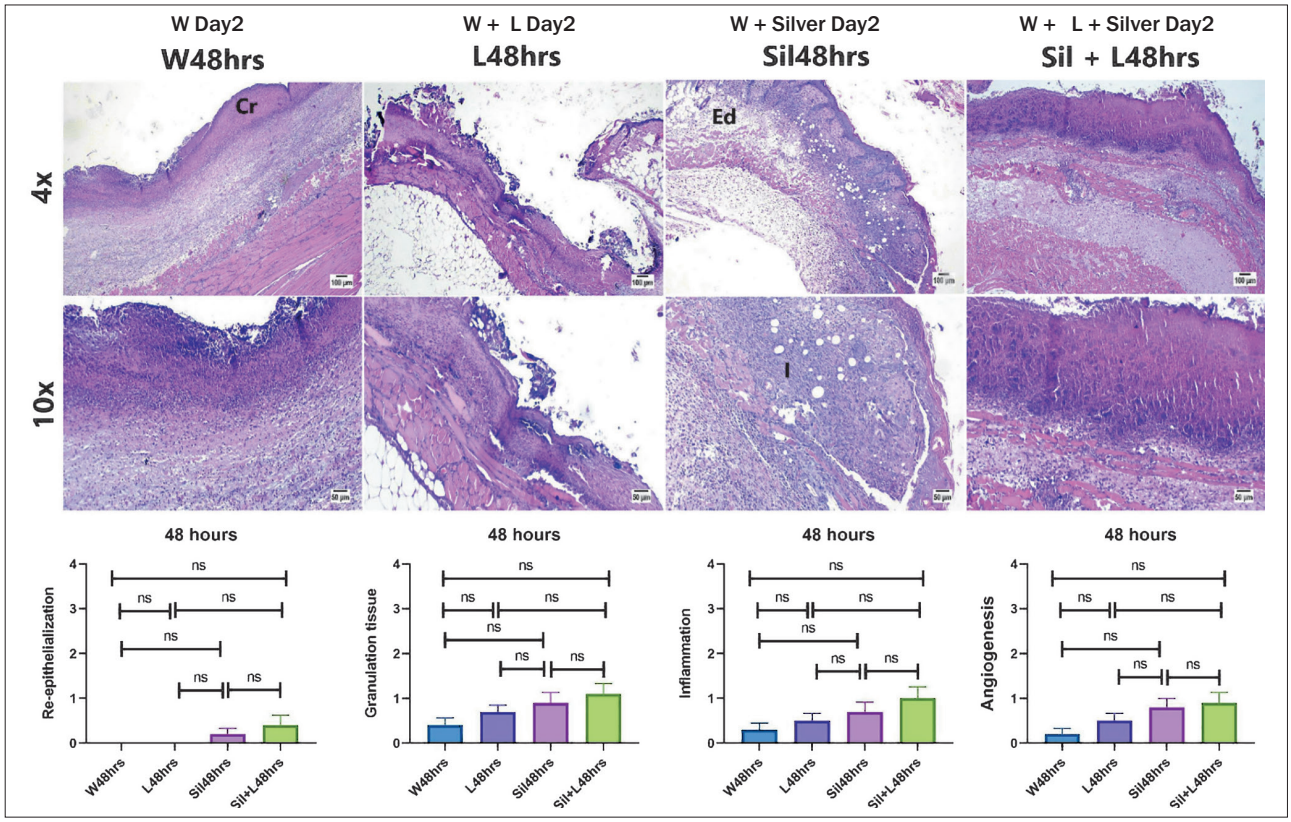

Рис. 3. Микрофотографии участков раны на 2-е сут после операции (во всех опытных группах отмечалось интенсивное острое воспаление, заполняющее раневую щель экссудатом и некротическими корочками; (Cr) корочка, (Ed) отек и (I) воспаление). Графики показывают гистопатологические параметры оценки заживления раны через 48 ч.
Fig. 3. Photomicrograph of wounded areas at day 2 post-surgery (all experimental groups displayed intense acute inflammation filling the wound gap with exudates and necrotic crusts covering; (Cr) crust, (Ed) edema, and (I) inflammation. Charts showing histopathological parameters of wound healing evaluation at 48 hours.

parameters high level of e-epithelialization, granulation tissue and angiogenesis with less inflammation score (Fig. 4).

W Day 21 and **L Day 21** groups exhibit inappropriate wound healing without epidermal covering. **Silver Day 21** and **L + Silver Day 21** groups showing compete epidermal growth and reduce the inflammation of the filling fibrovascular tissue.

Inflammatory Cytokines Assay

The data were entered, coded and analysed using the Statistical Package for the Social Science (IBM-SPSS,v.26). The experimental data were expressed as the mean ± standard error of the mean (SEM) using the ANOVA test, followed by a post-comparison test to determine which group had the best effect.

Serum TNF α

Tumor necrosis factor alpha (TNFα) levels increased significantly more in the **Wounded group** compared to the normal group ($p < 0.0001$), TNFα levels in the laser group and silver group had significantly lower concentrations than the injured group (positive control) at day 2 ($p < 0.0002$), while the combined group (**L+ Silver**) had the lowest significant concentration at day 2. Over time, the TNFα level decreased in all groups, reaching the trough level on day 21, and the combined group also had the highest significance compared to the injured group(untreated); The downregulation of the cytokine level during the experiment period showed at (Table 2).

Serum IL6

Table 3 displays the downregulation of interleukin 6 (IL6) level of during the inflammation stage and remodeling stage; levels increased significantly more in the **Wounded group** compared to the normal group ($p < 0.0001$), while IL6 levels in the laser group and silver group had significantly lower concentrations than the injured group (positive control) at day 2, while the combined group (**L + Silver**) had the lowest significant concentration at the same time (inflammation stage). Over time, the IL6 level decreased in all groups, Moreover the combined group also had the highest significance compared to the untreated group.

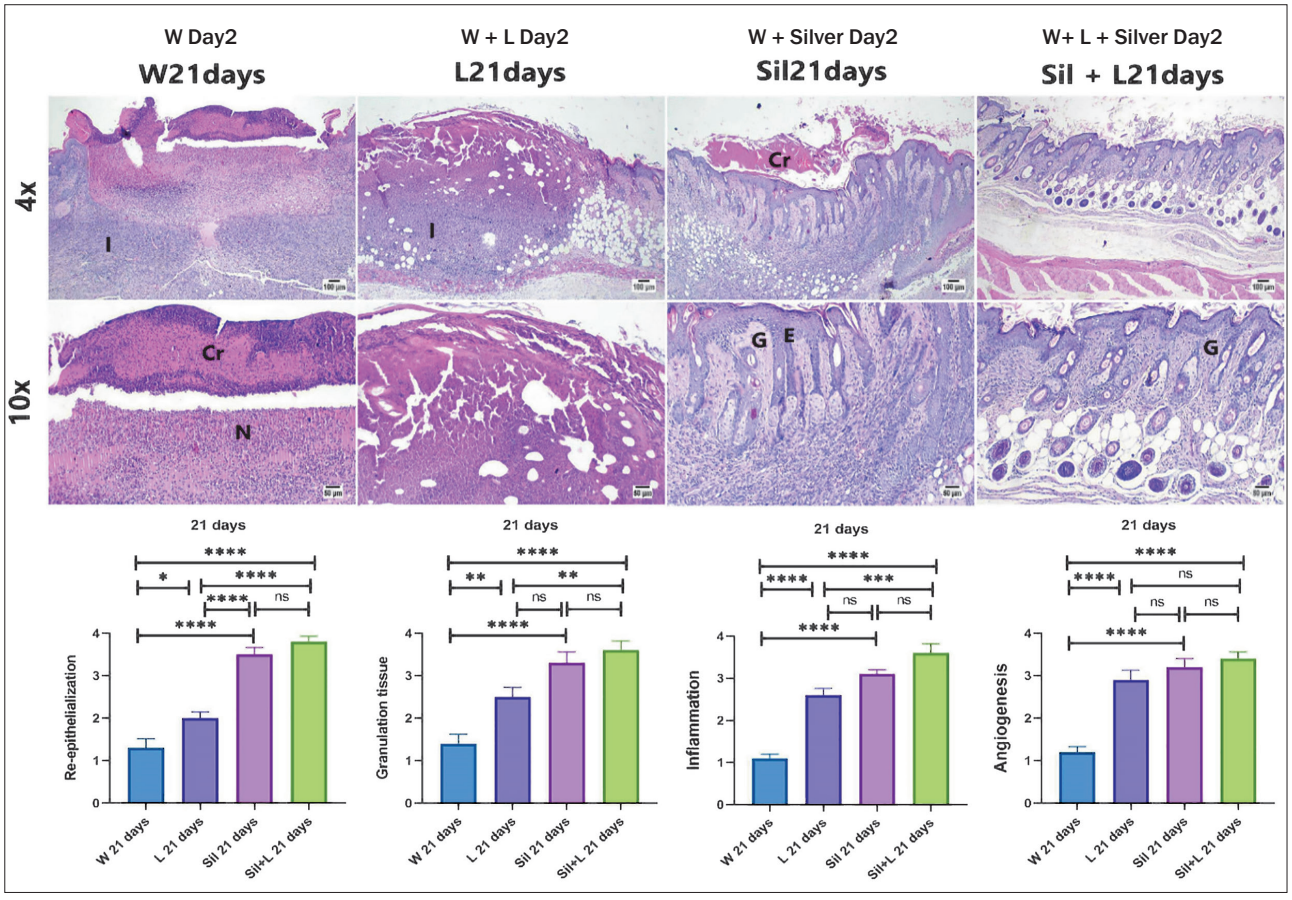

Рис. 3. Микрофотография участков раны на 21-е сут после операции. (E) Эпидермальный слой, (G) грануляционная ткань, (I) воспаление, (N) некроз. Графики показывают параметры оценки заживления раны на 21-е сут.
Fig. 4. Photomicrograph of wounded areas of different group at Day21 post-surgery. (E) Epidermal layer, (G) granulation tissue, (I) inflammation, (N) necrosis. Charts showing parameters of wound healing evaluation at day 21.

Serum IL1 β

Table 4 indicates that interleukin 1β (IL1β) levels increased significantly more in the Wounded group compared to the normal group (p <0.001), while IL1 β levels in the laser group and silver group had significantly lower concentrations than the injured group (positive control at day 2, while the combined group (**L+ Silver**) had the lowest significant concentration at day 2. Over time, the IL1 β level decreased in all groups, reaching the trough level on day 21, and the combined group also had the highest significance compared to the injured group**.**

Discussion

The natural process of wound healing entails a series of complicated cellular and biomolecular processes that restore damaged wound tissue into its original state when injury occurred [29]. During an injury, a blood clot is formed due to the damage in capillary and followed by the early phase of the inflammation, immune response to injury/wound or infection causes inflammation [31], infections related to various wounds, elevate medical expenditures and put a strain on health-care systems due to prolonged hospital admissions, treatment failures, infection persistence, and delayed wound

Таблица 2
Уровни TNFα (пг/мл) в сыворотке крови
Table 2
Serum levels of TNFα (pg/ml)

Группа Group	2 сут Day 2	21 сут Day 21
Группа без воздействия Normal	293.9 ±12.42	293.9 ±12.42
Рана Wounded	713.4 ±14.39****	486.9 ±15.27
Рана + низкоинтенсивный лазер Wounded + Low Level laser	581.2 ±31.84***	420.9±12.01
Рана + серебро Wounded + Silver	522.9± 26.62+++	389.1 ±23.44 ns
Рана + низкоинтенсивный лазер + серебро Wounded + Low Level laser + Silver	413.4± 17.34 **	300.6 ±21.7 ns
p value	<0.0001 ####	<0.0001 ++++
F	25.85	19.13

Среднее ± стандартная ошибка.
####: Достоверная разница между всеми группами на 2-е сут p < 0,0001.
++++: Достоверная разница между всеми группами на 21-е сут p < 0,0001.
****: Достоверная разница по сравнению с группой N при p < 0,0001.
***: Достоверная разница по сравнению с группой N при p < 0,0002.
+++: Достоверная разница по сравнению с группой N при p < 0,0005.
**: Достоверная разница по сравнению с группой N при p < 0,0027.
ns: Недостоверная разница по сравнению с группой N.

Means ± standard error.
####: Significant difference between all groups at day 2 p < 0.0001.
++++: Significant difference between all groups at day 21 p < 0.0001.
****: Significant difference in comparison with N group at p < 0.0001.
***: Significant difference in comparison with N group at p < 0.0002.
+++: Significant difference in comparison with N group at p < 0.0005.
**: Significant difference in comparison with N group at p < 0.0027.
ns: Non-Significant difference in comparison with N group.

Таблица 3
Уровни IL6 (пг/мл) в сыворотке крови
Table 3
Serum levels of IL6 (pg/ml)

Группа Group	2 сут Day 2	21 сут Day 21
Группа без воздействия Normal	51.09± 2.296	51.09± 2.296
Рана Wounded	303.1 ±18.32 ****	91.81 ±4.571 ns
Рана + низкоинтенсивный лазер Wounded + Low Level laser	203.1± 18.32 ***	71.22± 3.267 ns
Рана + серебро Wounded + Silver	160.8± 11.98 **	66.27 ±2.482 ns
Рана + низкоинтенсивный лазер + серебро Wounded + Low Level laser + Silver	128.3± 16.34 *	56.7± 2.134 ns
p value	<0.0001 ####	0.001 +++
F	19.58	35.85

Среднее ± стандартная ошибка.
###: Достоверная разница между всеми группами на 2-е сут p < 0,0001.
+++: Достоверная разница между всеми группами на 21-е сут p < 0,0001.
****: Достоверная разница по сравнению с группой N при p < 0,0001.
**: Достоверная разница по сравнению с группой N при p < 0,0052.
*: Достоверная разница по сравнению с группой N при p < 0,0256.
ns: Недостоверная разница по сравнению с группой N.

Means ± standard error.
###: Significant difference between all groups at day 2 p < 0.0001.
+++: Significant difference between all groups at day 21 p < 0.0001.
****: Significant difference in comparison with N group at p < 0.0001.
**: Significant difference in comparison with N group at p < 0.0052.
*: Significant difference in comparison with N group at p < 0.0256.
ns: Non-Significant difference in comparison with N group.

Таблица 4
Уровни IL1β (пг/мл) в сыворотке крови
Table 4
Serum levels of IL1β (pg/ml)

Группа Group	2 сут Day 2	21 сут Day 21
Группа без воздействия Normal	70.96± 4.937	70.96± 4.937
Рана Wounded	170±25.29***	65.77±4.9
Рана + низкоинтенсивный лазер Wounded + Low Level laser	141.9±21.28 *	62.22±3.2
Рана + серебро Wounded + Silver	102.5±22.76	59.91±6.269
Рана + низкоинтенсивный лазер + серебро Wounded + Low Level laser + Silver	80.92±20.45	52.34±3.9941
p value	0.04#	> 0.05
F	2.936	1.42

Среднее ± стандартная ошибка.
***: Достоверная разница по сравнению с группой N при p < 0,001.
*: Достоверная разница по сравнению с группой N при p < 0,012.
#: Достоверная разница в сравнении без групп на 2-е сут при p < 0,4.
ns: Недостоверная разница по сравнению с группой N.

Means ± standard error.
***: Significant difference in comparison with N group at p < 0.001.
**: Significant difference in comparison with N group at p < 0.012.
#: Significant difference in comparison within groups at day 2 at p < 0.04.
ns: Non-Significant difference in comparison with N group.

healing, which often leads to amputation and increased death [32].

The wound healing process involves multiple cellular and extracellular pathways through overlapping phases, namely hemostasis/inflammatory phase, proliferative phase, and remodelling phase [33]. The goal of the process is to restore tissue integrity and functions. Damaged blood vessels constrict during a vascular inflammatory response, and coagulation is brought on by thrombocytes congregating in a fibrin network. The wound then heals as a result of angiogenesis and re-epithelialization that take place during the proliferative stage. The remodelling phase involves reorganization, degradation, resynthesis of the extracellular matrix, and remodelling of the granulation tissue in order to achieve the maximum tensile strength [23].

Wound healing is strictly regulated by a number of cytokines and growth factors circulated at the wound site [34]. Decreased collagen deposition and growth factor production in wounds due to elevated levels of pro-inflammatory cytokines postpone wound healing [35]. Accelerating healing with minimal scars is the goal of most wound healing research [36].

Based on the experimental findings of the current study, the topical treatment application of silver nanoparticles, LLL alone or in combination gives great effect prevention of infections, decrease inflammation, complete healing and minimal scarring, moreover comparison of the rate of wound contraction among normal healing group (control) and other treated groups specially combined one, histological evaluation and other analysis which selected to monitor the progress showed a drastic difference in the healing pattern. The drastic decrease in wound contraction in control animals clearly indicated the need of therapeutic aid which could speed up the wound-healing process. Hence, in the current report, we aspired to understand the concept behind impaired wound healing in albino mice, and to come up with a therapeutic plan to accelerate healing in these persistent wounds using LLL and silver.

In the present study wound healing observed by morphological examination and photographed, wound closure in all wounded groups was measured every three days after injury till day 21, healing rate calculated [27] and give evidence that treated mice with AgNPs and/ or LLL as compared to the normal healing mice enhanced wound healing to reach 100% and 93%, respectively. Similar to our observation, Amini and his colleagues (2023) [37] have found that Ag-hydrogel nanocomposite groups consistently closed faster than control groups, and the original wound area in groups treated with Ag was significantly smaller at weeks 1, 2, and 3 post-wounding, and they confirming that Ag nanocomposite treatments accelerate the wound healing process.

AgNPs accelerate wound contraction and aid in the healing process by promoting fibroblast migration and stimulating fibroblast differentiation into myoblasts, as demonstrated by Tyavambiza et al. (2021) [38]. Also, due to the anti-inflammatory properties of AgNPs, the topical application of AgNPs in the wound area reduces the release of cytokines and lymphocytes, and reduces mast cell infiltration, which then promotes wound healing with minimal scarring [39]. Similarly, in other researches, AgNPs accelerated the rate of wound healing by activating the proliferation and migration of keratinocytes. Moreover, they aided in the differentiation of fibroblasts into myofibroblasts, which accordingly promoted wound contraction and speeded up the healing of severe wounds [38,40].

In terms of healing, the elucidation of pro-inflammatory and anti-inflammatory pathways is important for the development of strategies to defend regenerative tissue from damage caused by imbalances in cytokines, oxidants, antioxidants within the wound. Information about specific subsets of inflammatory

cell lineages and the cytokine network orchestrating inflammation associated with tissue repair has increased [40]. According to our data the treatment of wounded mice with silver and/or LLL produced a significant decrease of the elevate TNF-α, IL6 and IL1β levels as compared to the corresponding wounded controls, untreated group gave the highest significance and the highest level of the cytokine as compared to the normal levels- which illustrate the severe inflammation in the normal healing - after that cytokine level decrease to be near normal values, better values seen at the group treated with silver and LLL together.

Similar observations were obtained by Franková and his colleagues (2016) [41], who made an in vitro study by topical application of AgNPs in mice with burn wounds which results in decreasing counts of neutrophils and low levels of IL6, accompanied by an increase in the levels of IL10, TGF- β, vascular endothelial growth factor (VEGF), and interferon (IFN)- γ. They also have reported that AgNPs decreased the release of some pro-inflammatory cytokines and growth factors from normal human epidermal keratinocytes (NHEKs) and normal human dermal fibroblasts after 24 h at all the studied AgNPs concentrations (0.25, 2.5, and 25 μg/mL).

AT the present investigation the expression of TNF-α, IL6 and IL1ß in the laser group was seen in day 2 and decreased gradually till day 21.This may be due to the role of LLL on the surface epithelium cells (keratinocytes) to produce the pro-inflammatory cytokines which is needed in the acute inflammation during wound healing and also in a faster closure of the wound surface [42], after that the levels decreased gradually may occurred due to LLL anti-inflammatory effects which directly related to reduction of pro-inflammatory cytokines, as well as the amount of chemical mediators. The results indicate that LLL induces an inflammatory reaction that may modulate transcription factors linked to mRNA expression pro-inflammatory cytokines. These data are corroborated by previous studies which suggested that laser therapy can reduce the production of inflammatory mediators and events that contribute to balance the inflammation process [43].

The reason for the effective acceleration of wound healing using LLL was that the absorption of laser with specific wavelength by target tissue may result in the enhancement of fibroblast. proliferation and the promotion of collagen metabolism and granulation tissue formation also improvement of mechanical parameters and histopathological changes [42] which supported by present data. The major studies have suggested that either elements in the mitochondrial cytochrome system or endogenous porphyrins in the cells are the energy-absorbing chromophores in LLL [43,44]. It is important here to mention that photoenergy of 650nm wavelength at the given parameters possibly induced the fibroblasts to secrete the growth factors that probably acted in an autocrine manner to increase their rate of mitosis and or reduce cell death [44]. The response of low energy laser on cells may be dose dependent as well as wavelength dependent [44]. Therefore, correct energy density with an appropriate wavelength which can be easily and safely absorbed by the targeted tissues is strongly suggested.

Conclusion

Our results mandate the conclusion that the topical application of silver nanoparticles in combination with LLL following wounding had salutary effect on wound-healing progression, possibly through the decreasing of the inflammatory cytokines, activation of wound fibroblasts and elevation of collagen synthesis which accelerates wound healing rate. Testing the same optimal dose with the same power, center wavelength, laser spot size and duration, but with different doses of silver for its tissue regenerative potential is highly recommended. Although these results are very promising, more experimental studies for better understanding of silver and laser-assisted enhanced tissue regeneration are recommended.

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ORIGINAL ARTICLES

PHOTODYNAMIC THERAPY IN TREATMENT OF SQUAMOUS CELL CARCINOMA OF ORAL CAVITY WITH CHLORINE e6 PHOTOSENSITIZER WITH LONG-TERM FOLLOW UP

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Abstract

Photodynamic therapy is an effective method for treating superficial forms of malignant neoplasms, characterized by a minimal risk of damage to normal tissues. In this study, we presented our experience of treating cancer of the oral mucosa using photodynamic therapy, and analyzed the immediate and long-term results of treatment. 38 patients with squamous cell carcinoma of oral cavity mucosa, with a depth of invasion no more than 7 mm, were included in the study. All patients underwent photodynamic therapy with chlorine e6 based photosensitizer. Photosensitizers were administered intravenously 3 hours before irradiation, at a dosage of 1 mg/kg of the patient's weight. Photodynamic therapy was performed with the following parameters: P – 1.0 W, Ps – 0.31 W/cm^2, E – 300 J/cm^2. The area of one irradiation field ranged 1.0-2.0 cm^2. Treatment effect was evaluated by RECIST 1.1. Overall survival, cancer-specific survival, and disease-free survival were calculated using Kaplan-Meier curves. Evaluation of adverse events was made by CTCAE 5.0 criteria. At 35 (92.1%) out of 38 cases, complete regression was observed after photodynamic therapy. Among them in 3 out of 35 patients relapse was diagnosed in 11.5 to 43.2 months. The total number of patients who didn't respond to treatment was 6 (15.8%). Follow-up period was 4.2-87.3 months. (mean 42.9). 34 (89.5%) out of 38 patients are alive, 1 (2.6%) died from progression, and three died from other causes. The 5-year overall survival rate was 82.1%, cancer-specific survival rate was 97.0%, and disease-free survival rate was 81.1%. Among the factors significantly ($p < 0.05$) influencing relapse-free survival: depth of invasion < 5 mm (p – 0.013) and the presence of leukoplakia (p – 0.007). When assessing cancer-specific survival, factors worsening the prognosis were: age >70 years (p – 0.034) and the presence of leukoplakia (p – 0.007). Photodynamic therapy is an alternative treatment method of oral cancer superficial lesions, in case of proper assessment of primary lesion and in case of possibility of full irradiation of the tumor. Moreover, after using photodynamic therapy, the underlying connective-muscular structures are preserved, which promotes rapid healing with minimal scarring, the functions of the affected organ remain intact, and cosmetic defects do not form.

Key words: photodynamic therapy, squamous cell carcinoma of the oral cavity, leukoplakia, prognosis factors for oral cancer, survival rate for oral cancer.

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РЕЗУЛЬТАТЫ ЛЕЧЕНИЯ БОЛЬНЫХ РАКОМ ПОЛОСТИ РТА ПРИ ПОМОЩИ ФОТОДИНАМИЧЕСКОЙ ТЕРАПИИ С ФОТОСЕНСИБИЛИЗАТОРОМ НА ОСНОВЕ ХЛОРИНА e6

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Фотодинамическая терапия является эффективным методом лечения поверхностных форм злокачественных новообразований, характеризующимся минимальным риском повреждения нормальных тканей. В данном исследовании мы представили опыт лечения рака слизистой оболочки полости рта при помощи фотодинамической терапии, проанализировали непосредственные и отдаленные результаты лечения. В группу были включены 38 пациентов с плоскоклеточным раком слизистой оболочки полости рта с глубиной инвазии не более 7 мм. Всем пациентам выполнена фотодинамическая терапия с фотосенсибилизатором на основе хлорина е6. Фотосенсибилизатор вводили внутривенно за 3 ч до облучения, в дозировке 1 мг/кг веса пациента. Параметры облучения: плотность мощности на выходе волокна – 1,0 Вт, плотность мощности – 0,31 Вт/см^2, световая доза – 300 Дж/см^2. Площадь одного поля облучения составляла 1,0 – 2,0 см^2. Эффект от лечения оценивали по системе RECIST 1.1. Общая выживаемость, канцер-специфичная выживаемость и безрецидивная выживаемость были определены при помощи кривых Каплан-Майера. Оценка нежелательных явлений произведена по критериям CTCAE 5.0. У 35 (92,1%) из 38 пациентов получена полная регрессия опухолевого очага после ФДТ, из них рецидив заболевания выявлен у 3 из 35 пациентов в сроки от 11,5 до 43,2 мес. Общее количество пациентов, не ответивших на лечение, составило 6 (15,8%) человек. Общий период наблюдения пациентов составил 4,2-87,3 мес (в среднем 42,9 мес). 34 (89,5%) из 38 пациентов живы, 1 (2,6%) умер от прогрессирования заболевания, трое погибли по другим причинам. 5-летний показатель общей выживаемости составил 82,1%, канцер-специфичной выживаемости – 97,0%, безрецидивная выживаемость составила – 81,1%. Среди факторов достоверно ($p < 0,05$) влияющих на безрецидивную выживаемость: глубина инвазии < 5 мм (p 0,013) и наличие лейкоплакии (p 0,007). При оценке канцер-специфичной выживаемости факторами, ухудшающими прогноз, стали: возраст >70 лет (p 0,034) и наличие лейкоплакии (p 0,007). Фотодинамическая терапия является альтернативным методом лечения поверхностных очагов рака полости рта, при адекватной оценке распространенности первичного очага и возможности полноценного облучения опухоли. При этом после применения ФДТ сохраняются подлежащие соединительно-мышечные структуры, что способствует быстрому заживлению с минимальным рубцовым процессом, остаются сохранными функции пораженного органа и не формируются косметические дефекты.

Ключевые слова: фотодинамическая терапия, плоскоклеточный рак полости рта, лейкоплакия, факторы прогноза рака полости рта, выживаемость при раке полости рта.

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Introduction

Currently, the main method of treatment of oral squamous cell cancer (SCC) is surgery [1]. Surgical treatment allows adequate staging of the cancer process and identification of prognostically unfavorable factors requiring adjuvant treatment [2]. However, even in initial oral cavity SCC (T1-T2), a reconstructive-plastic stage is performed in 86%. According to a meta-analysis, in the surgical treatment of oral cavity SCC, small defects are replaced with local flaps in 45% of cases. If necessary, in 41%, microsurgical free flap transplantation is used [3]. Such extensive surgical interventions undoubtedly increase surgical trauma and rehabilitation time. The quality of speech, the act of swallowing deteriorates, and the cosmetic result is not always satisfactory, which in turn reduces the quality of life [4-6].

An alternative to surgical treatment of oral SCC is chemoradiation therapy in a stand-alone option [7]. The difficulties of radiation treatment of oral cavity SCC are associated with the fact that radiation eradication of the tumor usually requires the administration of total doses exceeding the tolerance of the surrounding normal tissues. This leads to adverse events such as mucositis, osteomyelitis, dysgeusia, hyposalivation, and radioinduced oncopathologies [8].

Photodynamic therapy (PDT) can be used as an independent technique for radical treatment of oral cavity SCC (T1-T2) with comparable antitumor results with conventional treatment methods. Many adverse events associated with surgical and chemoradiotherapy treatment are minimized or absent [9-13]. In a retrospective meta-analysis comparing the results of treatment of oral cavity SCC with surgery and PDT, the oncologic results were comparable, but in the PDT group there was a significant improvement in the quality of life [11]. The use of PDT is especially relevant in severe, somatically-challenged patients for whom other treatment methods are contraindicated. It is possible to achieve complete resorption of the primary focus and treatment of combined pathology of the oral mucosa –

leukoplakia [14]. The use of PDT does not limit the further use of traditional methods, such as surgical treatment, chemoradiation therapy or immunotherapy [15].

Photosensitizers (PS) based on the active substance chlorin e6 are licensed and actively used in the treatment of precancerous diseases, oncopathology of skin and mucous membranes [16, 17]. Chlorin e6 activation is achieved by local exposure to light radiation with a wavelength of 660-670 nm. This induces intracellular cytotoxic effects, such as the formation of free oxygen radicals, the effects of cellular hypoxia, and systemic immune response. The effective light penetration depth of PDT is approximately 10 mm [18]. This limits the use of PDT as a radical treatment for oral cancer with a depth of invasion (DI) of the primary focus greater than 5-7 mm [19, 20].

Numerous advantages of PDT, such as minimal toxicity of surrounding healthy tissues due to selective accumulation of PS in the tumor; absence of limiting doses of PS and light exposure and, as a consequence, the possibility of multiple repetition of the procedure; convenience of application in case of multiple lesions and better cosmetic results due to preservation of collagen fibers structure, which contributes to the formation of normotrophic scars; and the possibility of combination with other methods, make the PDT a valuable option [21, 22].

In a meta-analysis of 43 clinical trials of PDT for oral cancer, complete regression was observed in 94.4% and the 5-year survival rate was 84.2% [11]. Also, a comparison of surgical treatment and PDT in the initial stages of oral cancer (T1-T2) showed no significant superiority of either technique. The PDT group included 126 patients with T1 and 30 patients with T2 oral SCC, while the surgical group included 58 patients with T1 and 33 patients with T2, respectively. The complete tumor response to treatment, at T1 was 86% and 76% for PDT and surgery, respectively. At T2, it was 63% for PDT and 78% for surgery [9].

In another systematic meta-analysis including 900 patients with head and neck SCC, complete response to PDT was found in 741 cases (82%). Jiao Lin et al. argue that PDT is an effective technique for the treatment of superficial foci of head and neck SCC, but point out the need for optimization of treatment regimens and further studies to evaluate the efficacy of PDT [23].

In this clinical study, we present our own results of PDT use in T1-T2 stages of oral cavity SCC.

Materials and Methods

The prospective study included 38 patients who came to the A. Tsyb Medical Radiological Research Centre (MRRC) – the branch of the FSBI "National Medical Research Radiological Centre" (NMRRC) of the Ministry of Health of the Russian Federation from May 2016 to September 2023 for oral cavity SCC. All patients were diagnosed with primary oral cavity SCC (T1-T2). The main inclusion criterion was a depth of invasion (DI) of less than 7 mm. The DI of the primary focus was determined by ultrasound, CT and MRI (Fig. 1). Exclusion criteria were: tumor invasion depth more than 7 mm, presence of regional or distant metastases, technical impossibility to include all necessary tissue volume in the irradiation field due to anatomical features of the affected area.

There were 21 (55.3%) men and 17 (44.7%) women in the group, aged 37 to 83 (average 61) years at the time of treatment. Localization of the tumor process: lateral surface of the tongue – 16 (42.1%), floor of the oral cavity – 11 (28.9%), lip mucosa – 5 (13.2%), cheek – 3 (7.9%), alveolar process – 2 (5.3%), retromolar space – 1 (5.3%). The depth of invasion of the primary focus ranged from 0 (in situ cancer) to 7 mm (mean 3.4 mm), among them T1 – 28 (73.7%) tumors, of which 5 (13.2%) were cancer in situ and T2 – 10 (26.3%) cases. The depth of invasion was <0mm in 5 (13.2%) cases, 1-5 mm in 26 (68.4%) cases and 5-7 mm in 7 (18.4%) cases. Visible tumor sizes ranged from 2x3 to 35x15 mm, with the total tumor area ranging from 0.06 to 5.25 cm^2.

Characteristics of the primary focus was the following: superficial form – 24 (63,2%), ulcerated form – 4 (10,5%) cases, exophytic focus – 7 (15,8%), endophytic focus – 4 (10,5%) (Fig. 2). In 5 (13.2%) patients cancer developed on the background of leukoplakia (Fig. 3). General characteristics of the patients are presented in Table 1.

None of the patients were diagnosed with regional (cN0) and distant metastasis (cM0) according to clinical and instrumental examination. Staging was performed according to the UICC TNM system of 8th edition [24].

This clinical trial was approved by the local ethical committee of A. Tsyb MRRC (Ethical Committee Meeting Minutes No. 294 dated 11.07.2018). All patients signed informed voluntary consent.

Two chlorin e6-based preparations – photolon and photoran – were used as PSs, and were administered by intravenous drip at a dose of 1.0 mg/kg 3 h before PDT.

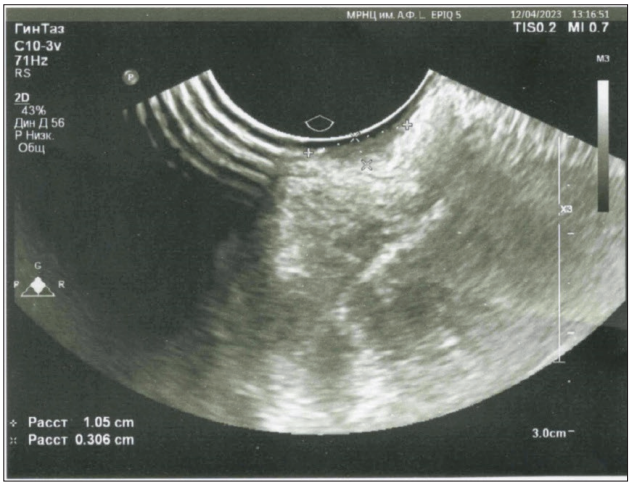

Рис. 1. УЗ отображение опухоли языка с определением глубины инвазии.
Fig. 1. US of tongue cancer with depth of invasion.

Таблица 1
Клинические характеристики пациентов
Table 1
Patient's clinical characteristics

Характеристика Characteristic	Количество пациентов (%) Number of patients (%)
Пол: Gender: Мужской Male Женский Female	 21 (55,3%) 17 (44,7%)
Возраст: Age: 30–59 60–69 70–79 80–89	 13 (34,2%) 17 (44,7%) 6 (15,8%) 2 (5,3%)
Локализация опухоли: Tumor localization Язык (боковая поверхность) Tongue (lateral surface) Дно полости рта Floor of the mouth Слизистая губы Mucous lips Щека Cheek Альвеолярный отросток Alveolar ridge Ретромолярное пространство Retromolar region	 16 (42,1%) 11 (28,9%) 5 (13,2%) 3 (7,9%) 2 (5,3%) 1 (2,6%)
Т категория T stage T1 T2	 28 (73,7%) 10 (26,3%)
Глубина инвазии Depth of invasion <0 (опухоль не определяется) <0 (tumor not detected) ≥1mm ≤5mm >5mm ≤7mm	 5 (13,2%) 26 (68,4%) 7 (18,4%)
Площадь опухоли: Tumor area: <1cm^2 1-2 cm ^{2} ≥2 cm^2	 25 (65,8%) 8 (21,1%) 5 (13,2%)
Тип опухоли: Tumor type: Поверхностная Superficial Язвенная Ulcerative Экзофитная Exophytic Эндофитная Endophytic	 24 (63,2%) 4 (10,5%) 6 (15,8%) 4 (10,5%)
Фоновое заболевание: Background disease: Лейкоплакия Leukoplakia Отсутствует Absent	 6 (15,8%) 33 (84,2%)

Irradiation was performed in a darkened room using a remote laser device "Latus-2" (wavelength 662 nm) with flexible quartz light guides (Fig. 4).

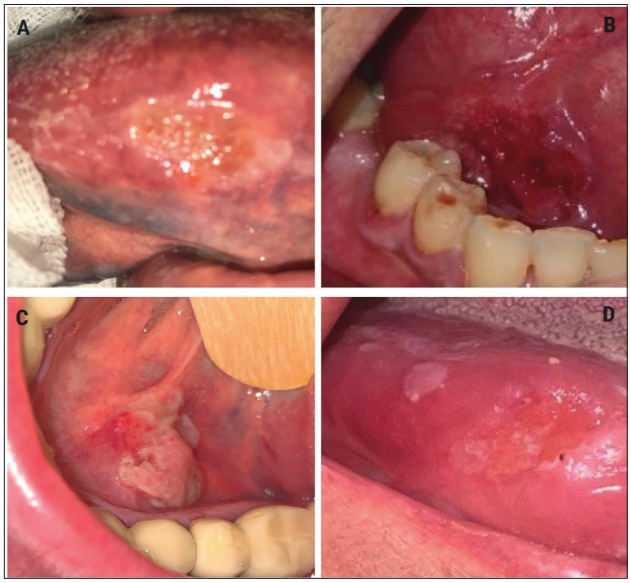

Рис. 2. Типы очагов: a – поверхностный; b – язвенный; c – экзофитный; d – эндофитный.
Fig. 2. Types of lesions: a – superficial; b – ulcerative; c – exophytic; d – endophytic.

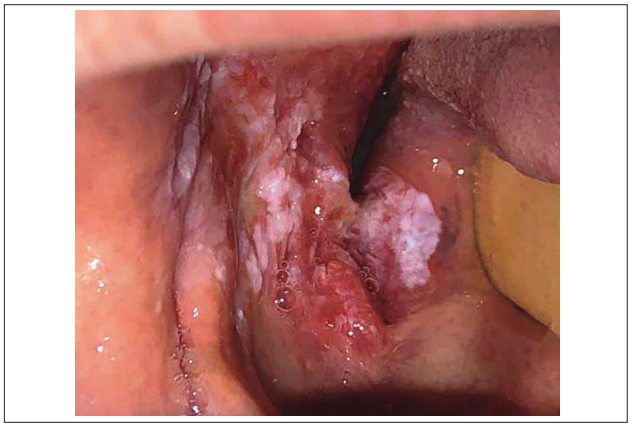

Рис. 3. Плоскоклеточный рак полости рта на фоне лейкоплакии.
Fig. 3. Squamous cell carcinoma of oral cavity with leukoplakia.

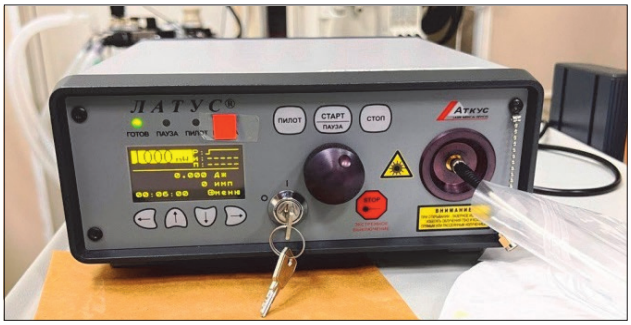

Рис. 4. Источник лазерного излучения аппарат «Латус-2» (662 нм).
Fig. 4. Source of laser irradiation «Latus-2» (662 nm).

The irradiation field included an area 5-10 mm away from the visible edges of the tumor mass. Surrounding tissues were covered with dark light-tight material to protect from damage to healthy tissues during PDT session (Fig. 5).

PDT parameters: power at the fiber output – 1.0 W, power density – 0.31 W/cm^2, light dose – 300 J/cm^2. The area of one irradiation field was 1.0 – 2.0 cm^2. In case of a large tumor focus size or presence of concomitant pathology in the form of leukoplakia, irradiation with two or more fields was performed in order to include all necessary tissues in the irradiation volume.

PDT was performed under local or general anesthesia if it was necessary to perform surgical interventions on the pathways of regional lymph drainage (biopsy of a sentinel lymph node or prophylactic lymphadenectomy).

Within 36 h after PS injection patients avoided direct sunlight and were in a darkened room (under the light not more than 1000 lux). A follow-up after the PDT in the clinic was from 3 to 7 days.

Adverse events were evaluated according to the CTCAE 5.0 criteria. Evaluation for adverse events was performed during the first 5 days after PDT and subsequently, during follow-up examinations at 4 weeks, 3, 6, 12 months and then once a year.

The first clinical and instrumental examination was performed 4 weeks after PDT with evaluation of the immediate response. In case of suspicion of residual tumor presence, biopsy was performed. Thereafter, patients underwent routine examination at 3, 6, and 12 months, respectively, and once a year thereafter.

The immediate response to treatment was evaluated as follows: complete response (CR) – complete regression of tumor focus, partial response (PR) – presence of residual tumor tissue in the PDT zone, disease progression (DP) – tumor enlargement after treatment. The distant result was evaluated in terms of more than 3 months with previously achieved CR on treatment. The distant result included: local recurrence (LR) – recurrence in the area of primary focus, regional recurrence (RR) – metastases to lymph nodes of the neck, distant metastases (DM).

Overall survival (OS), cancer-specific survival (CSS), and recurrence-free survival (RFS) were determined using Kaplan-Meier curves. Both progression by primary focus and progression by locoregional and distant metastases were considered in the analysis of RFS. Log-rank analysis was also performed to identify prognostically unfavorable factors. The analysis included: sex, age, tumor localization, tumor size, tumor type, and presence of background disease. Statistical significance was determined at $p < 0.05$.

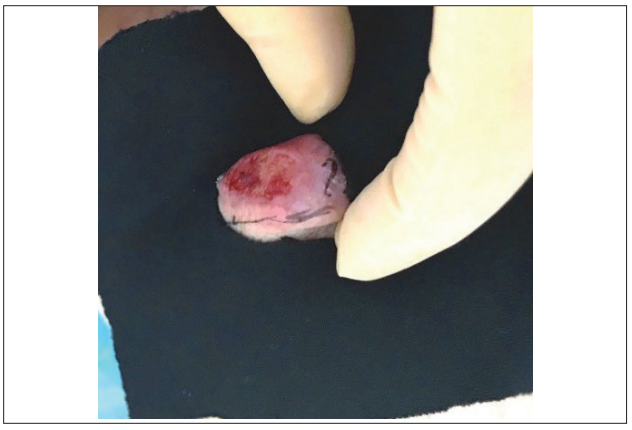

Рис. 5. Опухолевый очаг на нижней губе и защитный экран для здоровых тканей.
Fig. 5. Tumor lesion of lower limb with protective screen.

Results

Effectiveness of PDT

Initial signs of necrosis after PDT were observed immediately after the procedure, in the form of demarcation of the treatment zone due to vascular stasis. On 3-6 days after PDT a necrotic scab was formed, which was independently rejected on 10-15 days. Further, independent wound healing was observed within 1 month (Fig. 6).

At the first follow-up examination, 3 months later, 35 (92.1%) of the 38 patients had CR, and three patients had PR (7.9%). During the follow-up period, 3 of the 35 patients who had previously reported immediate CR had a recurrence between 11.5 and 43.2 months after treatment. In one case, the recurrence was both in the primary site and metastasis to regional lymph nodes,

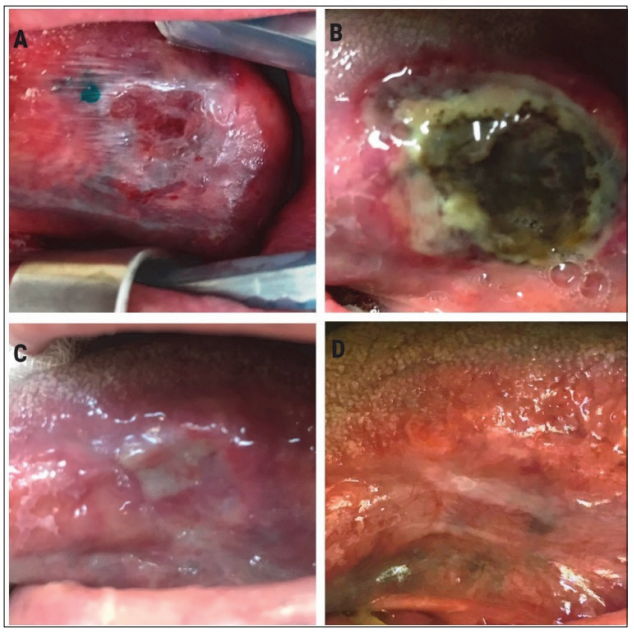

Рис. 6. Клинические изменения в зоне проведения ФДТ: a – через 10 мин после ФДТ; b – 5 сут после ФДТ; c – 20 сут после ФДТ; d – 3 мес после ФДТ.
Fig. 6. Clinical changings in PDT zone: a – 10 min after PDT; b – 5th day after PDT; c – 20th day after PDT; d – 3 months after PDT.

in another case the recurrence was only in the primary site, and in the third patient only regional recurrence was detected. Thus, the total number of patients who did not respond to treatment was 6 (15.8%). All 6 patients who did not respond to treatment underwent surgical treatment followed by adjuvant chemoradiation therapy. The total patient follow-up period was 4.2-87.3 months (mean 42.9 months), during this period 34 (89.5%) of 38 patients were alive, 1 (2.6%) died of disease progression, and three died of causes unrelated to oral cancer (Table 2).

At 5 years after treatment, the overall survival rate (OS) was 82.1%, the cancer-specific survival rate (CSS) was 97.0%, and the disease-free survival rate (DFS) rate was – 81.1% (Fig. 7).

We used log-rank analysis of such clinical and demographic factors as sex, age, focus location, T category, DI of the primary tumor, tumor area and type,

Таблица 2
Результаты лечения методом ФДТ
Table 2
Results of treatment by PDT

Клиническая характеристика Clinical characteristics	Количество пациентов (%) Number of patients (%)
Непосредственный ответ на лечение: Immediate response to treatment:	
Полный ответ (ПО) Full response (FR)	35 (92,1%)
Частичный ответ (ЧО) Partial response (PR)	3 (7,9%)
Прогрессия заболевания (ПЗ) Disease progression (PD)	0
Отдаленные результаты: Long-term results:	
Локальный рецидив (ЛР) Local recurrence (LR)	2 (5,3%)
Регионарный рецидив (РР) Regional recurrence (RR)	2 (5,3%)
Отдаленные метастазы (ОМ) Distant metastases (DM)	0
Причины смертности: Causes of mortality:	
Смерть от прогрессии Death by progression	1 (2,6%)
Другие причины Other reasons	3 (7,9%)
Лечение неполного ответа/рецидива: Treatment of incomplete response/relapse:	
Хирургия (первичный очаг) Surgery (primary site)	5 (13,2%)
Хирургия (шейная диссекция) Surgery (neck dissection)	2 (5,3%)
Лучевая терапия Radiation therapy	6 (15,8%)
Паллиативная химиотерапия Palliative chemotherapy	1 (2,6%)

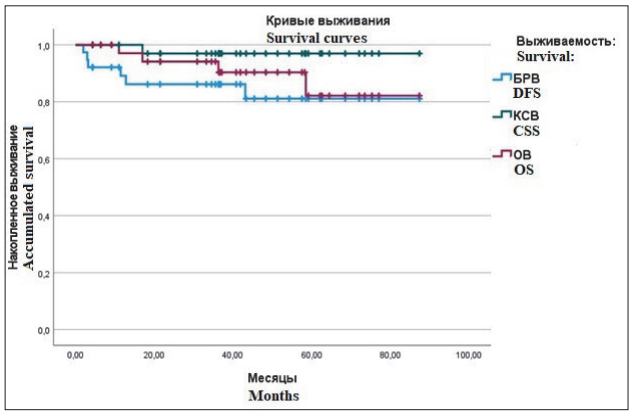

Рис. 7. Кривые Каплан-Майера по анализу общей выживаемости (ОВ), безрецидивной выживаемости (БРВ) и канцер-специфичной выживаемости (КСВ).
Fig. 7. Kaplan-Mayer curves in overall survival (OS) rate analysis, disease-free survival rate (DSS) и cancer-specific survival rate (CSS).

and the presence of leukoplakia to identify reliable prognostic factors in terms of OS, CSS, and DFS.

Statistical analysis revealed no significant ($p < 0.05$) difference in overall survival according to any clinical and demographic characteristics.

However, a significant difference was detected when analyzing cancer-specific survival for patients over 70 years of age. The 5-year CSS for patients over 70 years of age was 87.5%, versus 100% for those under 70 years of age (p 0.034). Another significant prognostic factor for worsening CSS was the presence of leukoplakia. The 5-year cancer-specific survival rates were 83.3% in the presence and 100% in the absence of leukoplakia (p 0.007).

In the analysis of disease recurrence, the presence of leukoplakia and depth of invasion were significant factors for prognosis. Thus, the recurrence-free survival rate was 57.1% for invasion depths greater than 5 mm versus 90.3% for invasion depths of 0 to 5 mm (p 0.013). The 5-year recurrence-free survival rate was 100% in patients with cancer in situ, in whom no invasive growth was detected according to the examination data (invasion depth 0 mm).

The second factor of recurrence was the presence of leukoplakia. In the presence of leukoplakia, 50% of patients developed recurrence after treatment, in its absence – the risk of recurrence was 90.6% (p 0.007) (Fig. 8-11). The summary data on DFS are summarized in Table 3.

Adverse events

The most common early adverse event was pain in the PDT area. This adverse event was observed in 35 out of 38 patients (92,1%) with various degrees of severity. The majority (25 out of 35) had insignificant soreness (grade 1), which did not affect daily activity and did not require drug correction. Seven of 35 patients had

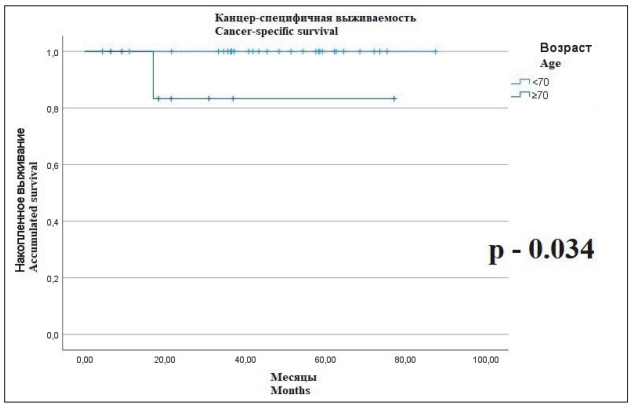

Рис. 8. Кривые Каплан-Майера по оценке возрастного фактора на канцер-специфичную выживаемость.
Fig. 8. Kaplan-Mayer curves in evaluation of age factor, affecting to cancer-specific survival.

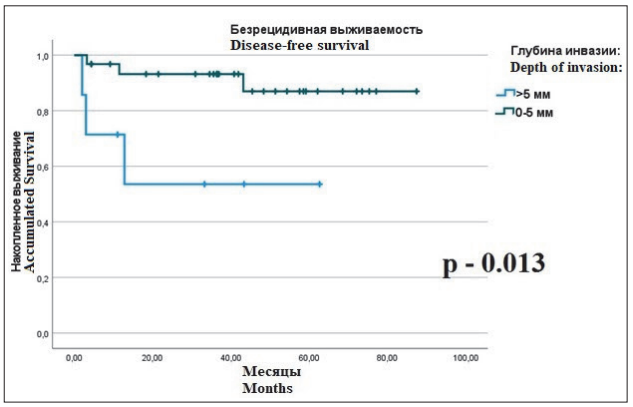

Рис. 10. Кривые Каплан-Майера по оценке фактора глубины инвазии на безрецидивную выживаемость.
Fig. 10. Kaplan-Mayer curves in evaluation of depth of invasion, affecting to disease-free survival rate.

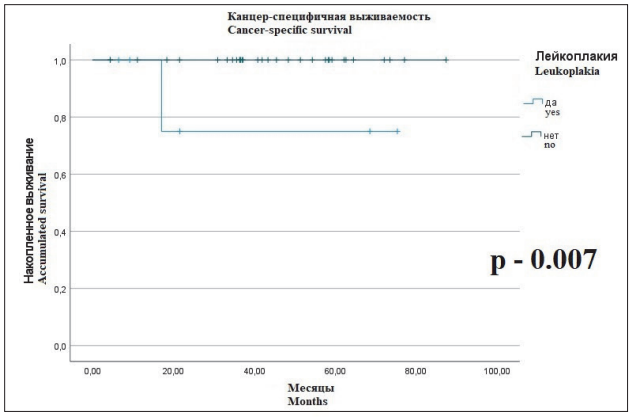

Рис. 9. Кривые Каплан-Майера по оценке фактора наличие лейкоплакии на канцер-специфичную выживаемость.
Fig. 9. Kaplan-Mayer curves in evaluation of leukoplakia present, affecting to cancer-specific survival.

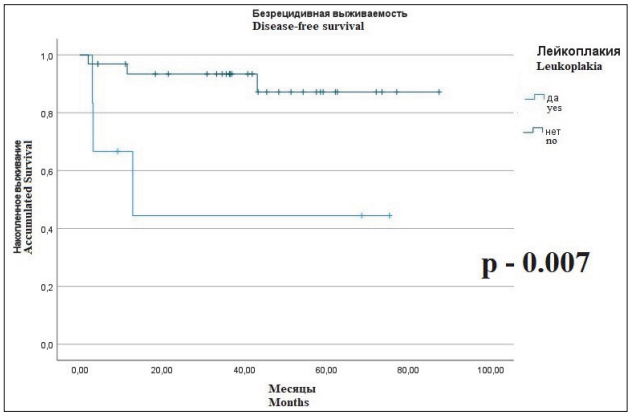

Рис. 11. Кривые Каплан-Майера по оценки наличие лейкоплакии на безрецидивную выживаемость.
Fig. 11. Kaplan-Mayer curves in evaluation of leukoplakia present, affecting to disease-free survival rate.

moderate pain (grade 2), which was managed with non-steroidal anti-inflammatory drugs. Severe pain (grade 3) was noted in 3 cases, requiring the use of narcotic drugs. This adverse event started on the 1-2 day after PDT and was resolved on the 6-10 day.

Another undesirable phenomenon was local edema of oral mucosa in the PDT area, observed in 50% of cases, in 19 out of 38 patients. Mainly (in 11 out of 19 patients) the edema was insignificant (grade 1), not affecting the functionality of the organ and did not require medication correction. In 8 out of 19 cases the edema was moderate (grade 2). Glucocorticosteroid administration in the postoperative period was required to restore the nutritional function and to stop the threat of respiratory failure through the upper respiratory tract. This phenomenon started on the 1st day after PDT and resolved on the 5th-6th day (Table 4).

In the treatment of mucosal cancer of the alveolar outgrowth in the area of PDT, bare bone site was observed in the area of PDT, with independent epithelization observed at 3-6 months after PDT. There were no other adverse events, including phototoxicity associated with PDT.

Таблица 4
Нежелательные явления после ФДТ
Table 4
Adverse events after PDT

Нежелательные явления Adverse events	Количество пациентов (%) Number of patients (%)
Боли в области ФДТ: Pain in the PDT area: Grade 1 Grade 2 Grade 3	35 (92,1%) 25 (65,8%) 7 (18,4%) 3 (7,9%)
Отек слизистой полости рта: Swelling of the oral mucosa: Grade 1 Grade 2	19 (50%) 11 (28,9%) 8 (21,1%)

Discussion

Our study on the use of PDT with chlorine-type PCs in T1-T2 stages of oral cancer showed good functional and aesthetic results of treatment, without mucosal scar

Таблица 3
Анализ безрецидивной выживаемости, в зависимости от клинических характеристик
Table 3
Analysis of disease-free survival according to clinical characteristics

Характеристика Characteristic	1-летняя безрецидивная выживаемость 1st year disease-free survival	2-летняя безрецидивная выживаемость 2nd year disease-free survival	P значимость (логарифмический тест) P significance (log test)
Пол: Gender: Мужской Male Женский Female	 90,45 (19/21) 84,6 (11/13)	 85,7 (18/21) 81,8 (9/11)	 0.757
Возраст: Age: <70 ≥70	 92,6 (25/27) 71,4 (5/7)	 88,9 (24/27) 60,1 (3/5)	 0.274
Локализация опухоли: Tumor localization Язык (боковая поверхность) Tongue (lateral surface) Дно полости рта Floor of the mouth Слизистая губы Mucous lips Щека Cheek Альвеолярный отросток Alveolar ridge Ретромолярное пространство Retromolar space	 84,6 (11/13) 81,8 (9/11) 100 (5/5) 100 (2/2) 100 (2/2) 100 (1/1)	 84,6 (11/13) 72,7 (8/11) 100 (5/5) 100 (1/1) 100 (2/2) 0 (0/1)	 0.332
Т категория: T stage: T1 T2	 91,7 (22/24) 80 (8/10)	 91,3 (21/23) 66,7 (6/9)	 0.184
Глубина инвазии: Depth of invasion: <0 (опухоль не определяется) <0 (tumor not detected) 1-5 mm 5-7 mm	 100 (4/4) 91,7 (22/24) 66,7 (4/6)	 100 (3/3) 91,3 (21/23) 50 (3/6)	 0.038
Площадь опухоли: Tumor area: <2 cm^2 ≥2 cm^2	 89,7 (26/29) 80 (4/5)	 85,2 (23/27) 80 (4/5)	 0.887
Тип опухоли: Tumor type: Поверхностная Superficial Язвенная Ulcerative Экзофитная Exophytic Эндофитная Endophytic	 100 (21/21) 66,7 (4/6) 66,7 (2/3) 75 (3/4)	 94,7 (18/19) 66,7 (4/6) 66,7 (2/3) 75 (3/4)	 0.329
Фоновое заболевание: Background disease: Лейкоплакия Leukoplakia Отсутствует Absent	 60 (3/5) 93,1 (27/29)	 40 (2/5) 92,6 (25/27)	 0.007

formation (Fig. 6C). Presumably this result is due to the preservation of tissue matrix components (collagen and elastin), while cellular vascular elements are damaged by PDT. It is believed that preservation of tissue architecture provides the best conditions for normal tissue regeneration and leads to superior healing with less scar formation [25, 26].

Of 38 patients with stage T1-T2 of oral mucosal cancer, complete response to the treatment was obtained in 35 (92.1%) cases up to 3 months after treatment. The obtained 5-year overall and cancer-specific survival rates were 82.1% and 97.0%, respectively, which is a reasonably good result. Such treatment results are comparable to those of PDT treatment with other PSs (complete response rate of 88.2%), surgery (5-year overall survival rate of 69.7-93.8%), and radiation therapy (5-year overall survival rate of 51.5-84.0%) [10, 27, 28]. Gluckman [29] and Biel [30] reported local recurrence in 16-36% after achieving a complete response in oral cancer for which PDT was performed. In our study, 2 out of 35 patients who achieved a complete response to treatment developed local recurrence. The risk of developing local recurrence was 5.7% after complete response. The overall 5-year recurrence-free survival rate was 81.1%.

We also investigated various clinical and demographic characteristics to identify unfavorable prognostic factors for recurrence. PDT is usually used for relatively small tumor foci. In our study, we primarily relied on the depth of tumor invasion rather than the area of the primary focus. Thus, the study included patients with superficial, relatively large (up to 5.3 cm^2) tumor foci, but with a shallow depth of invasion (up to 7 mm). At the same time, the data analysis did not show a statistically significant difference in the development of recurrence depending on the area of the primary focus. Thus, one patient in the distant period developed a relapse (at 43.2 months) in the area adjacent to the site of previously performed PDT. Thus, recurrence can develop regardless of the size of the primary focus. In such cases differential diagnostics between the primary tumor recurrence and the development of synchronous oncopathology of the oral cavity is difficult. Such a fact is explained by the so-called "malignization field" theory described by Slaughter in 1953 [31]. This theory assumes that squamous cell cancer does not arise from an isolated cell, but rather as a tendency towards anaplastic processes involving many cells simultaneously. This theory is partly supported by the fact that in our study one of the proven prognostic factors for recurrence was the presence of leukoplakia as a facultative precancerous lesion characterized by cellular dysplasia (Table 3) [32]. Nevertheless, PDT may be the treatment of choice for multifocal, superficial tumors because it can be repeated as often as necessary without loss of normal tissue functionality and without accumulation of toxic effects. In addition, conventional treatment remains as an option [26, 33, 34].

PDT has a high selectivity of PS accumulation in tumor tissues, which leads to minimal damage to healthy tissues. According to most researchers [35-37], the selectivity of PS accumulation in tumors with respect to healthy tissue can range from 2:1 to 15:1. In the cell, PSs mainly accumulate in mitochondria and lysosomes [38]. Minimal accumulation in the cell nucleus avoids the development of genetically therapy-resistant cells [39].

Since the 8th revision of TNM [24], it is the depth of invasion rather than the size of the primary focus that has become the determining factor in assessing the incidence and prognosis of oral cancer. In our study, it was also statistically significantly shown that the risk of recurrence increased with invasion depth greater than 5 mm. This fact is explained by the physical properties of PDT laser radiation, the penetrating ability of which is limited [40].

Conclusion

This study has shown that PDT with PS of chlorine series is an alternative method of treatment of superficial foci of oral cavity cancer in case of adequate assessment of the primary focus prevalence and possibility of full-fledged tumor irradiation. In this case, after PDT application, the underlying connective-muscular structures are preserved, which contributes to rapid healing with minimal scarring, the functions of the affected organ remain intact, and no cosmetic defects are formed.

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PHOTODYNAMIC THERAPY OF LEUKOPLAKIA OF THE ORAL MUCOSA: EXPERIENCE OF USING THE METHOD IN 223 PATIENTS

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Abstract

The aim of this study was to analyze the immediate and long-term results of using photodynamic therapy (PDT) in patients with leukoplakia of the oral mucosa. The retrospective study included 223 patients with morphologically verified leukoplakia of the oral mucosa. Patients received treatment at the hyperthermia and photodynamic therapy department from 2013 to 2023. The average age was 51.9±8.9 years. Clinically, 211 patients (94.6%) had a flat form of the disease, 12 patients (5.4%) had a verrucous form. The photosensitizer (PS), based on chlorin e6, was administered intravenously once in doses of 1.7 to 2.5 mg/kg body weight. Irradiation of pathologically changed lesions was carried out 2-2.5 hours after the end of its infusion, using a semiconductor laser device "UPL PDT", with a wavelength λ=665±5 nm. The laser radiation dose density varied from 25 to 100 J/cm^2, the power density from 0.07 to 0.32 W/cm^2, the duration of one irradiation field - from 2 to 13.5 minutes, depending on its linear dimensions. The severity of adverse reactions was assessed on days 1-30 after treatment using the CTCAE 4.03 scale. The immediate results of treatment were assessed up to 3 months after PDT, with morphological confirmation of the response to treatment. No serious adverse reactions were observed during PS infusion and irradiation. No symptoms of dermal phototoxicity were reported. During control examinations, up to 3 months after irradiation in patients with flat and verrucous forms, the frequency of complete regressions was 97.1% (n=205) and 58.3% (n=6) cases, partial regressions - 2,9% (n=7) and 25% (n=3) of cases, respectively. Objective response rates were observed at 100% and 83.3%, respectively. The period of observation of patients varies from 3 to 120 months. (average 66 months). The frequency of disease relapses during this period was 9%. Patients with partial regression and identified relapse were treated with repeated PDT sessions. PDT is a well-tolerated and effective treatment method for patients with leukoplakia of the oral mucosa, which has significant advantages over traditional methods of treating this precancerous pathology. These include minimal toxicity to the normal tissues surrounding the pathological foci due to the selective accumulation of PS in leukoplakia tissues, a slight risk of serious adverse reactions, the possibility of an outpatient session, the possibility of repeated treatment over a large area of damage and good cosmetic results).

Key words: photodynamic therapy, photolon, patients, leukoplakia of the oral mucosa, precancer.

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ФОТОДИНАМИЧЕСКАЯ ТЕРАПИЯ ЛЕЙКОПЛАКИИ СЛИЗИСТОЙ ПОЛОСТА РТА: ОПЫТ ПРИМЕНЕНИЯ МЕТОДА У 223 ПАЦИЕНТОВ

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Резюме

Целью настоящего исследования было изучение непосредственных и отдаленных результатов применения метода фотодинамической терапии (ФДТ) у пациентов с лейкоплакией слизистой оболочки полости рта (СОР). В ретроспективное исследование включено 223 пациента с морфологически верифицированной лейкоплакией слизистой оболочки полости рта. Пациенты получали лечение на базе отделения гипертермии и фотодинамической терапии в период с 2013 г. по 2023 г. Средний возраст составил 51,9±8,9 года. Клинически у 211 пациентов (94,6%) была плоская форма заболевания, у 12 пациентов (5,4%) – веррукозная. В качестве фотосенсибилизатора (ФС) использовался препарат фотолон, на основе хлорина е6, который вводился однократно внутривенно в дозах от 1,7 до 2,5 мг/кг массы тела. Облучение патологически измененных очагов осуществлялось через 2-2,5 ч после окончания его инфузии, с помощью полупроводникового лазерного устройства «УПЛ ФДТ» с λ=665±5 нм. Плотность дозы лазерного излучения варьировала от 25 до 100 Дж/см^2, плотность мощности от 0,07 до 0,32 Вт/см^2, продолжительность одного поля облучения – от 2 до 13,5 мин в зависимости от его линейных размеров. Степень выраженности нежелательных реакций оценивали на 1-30 сут после лечения по шкале

CTCAE 4.03. Непосредственные результаты лечения оценивались в сроки до 3 мес после ФДТ, с морфологическим подтверждением ответа на лечение. Серьезных нежелательных реакций при инфузии ФС и облучении не отмечено. Симптомов кожной фототоксичности не было зарегистрировано. При контрольных обследованиях, в сроки до 3 мес после облучения у пациентов с плоской и верукозной формами частота полных регрессий составила 97,1% (n=205) и 58,3% (n=6), частичных регрессий – 2,9% (n=7) и 25% (n=3) соответственно. Частота объективных ответов наблюдалась в 100% и 83,3% случаев соответственно. Период наблюдения за пациентами варьирует от 3 до 120 мес (в среднем 66 мес). Частота рецидивов заболевания за указанный период составила 9%. Пациенты с частичной регрессией и выявленным рецидивом получали лечение в объеме повторных курсов ФДТ. ФДТ является хорошо переносимым и эффективным методом лечения пациентов с лейкоплакией СОР и обладает весомыми преимуществами перед традиционными методами лечения данной предопухолевой патологии. К таковым можно отнести минимальную токсичность для окружающих патологические очаги нормальных тканей, незначительный риск возникновения серьезных нежелательных реакций, возможность амбулаторного проведения лечения, возможность многократного проведения лечения при большой площади поражения и хорошие косметические результаты.

Ключевые слова: фотодинамическая терапия, фотолон, пациенты, лейкоплакия слизистой полости рта, предрак.

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Introduction

In recent years, there has been a steady increase in the incidence of patients with precancerous diseases of various locations. One of the most common nosological forms of this pathology is leukoplakia, which is a chronic lesion of the mucous membranes, characterized by varying degrees of keratinization of the integumentary epithelium. Particular attention is paid to leukoplakia of the oral mucosa. According to S. Zhang et al., who analyzed 69 studies conducted in 28 countries, including a total of 1,263,028 potentially healthy people, leukoplakia in these locations was detected on average in 1.39% of cases (from 0.12% to 33. 33%) [1]. According to Y. Huang et al., the incidence of leukoplakia of the oral mucosa in the world is 4.1% [2].

This disease can be caused by exogenous and endogenous factors. Exogenous factors include smoking, eating spicy or sour foods, excessively hot food. alcoholic beverages; chronic mechanical damage to the oral cavity, etc. Endogenous factors include gastrointestinal diseases; hypovitaminosis A; diseases associated with metabolic disorders; chronic inflammatory processes in the oral cavity; neurodystrophic changes in mucous membranes [3].

According to A.L. Mashkilleison's classification, a distinction is made between flat, verrucous, erosive-ulcerative, mild leukoplakia and Tappeiner's leukoplakia (nicotine stomatitis), which have different malignancy potentials. According to H.M. Chen et al., the frequency of malignancy of the flat form varies from 1% to 7%, while for the verrucous form this figure increases significantly and averages 18-47% [4]. Based on an analysis of data on 16,604 patients, J. M. Aguirre-Urizar et al. report that this indicator, depending on the form of the disease, decreases from 1.1% to 40.8% [5].

Treatment of the oral mucosa leukoplakia depends on the clinical form of the disease, area of damage and a number of other reasons [6]. Thus, for a flat form, mainly conservative treatment is used, leading to regression of pathological foci. In this case, the termination of irritating factors is a prerequisite. Local treatment includes sanitation of the oral cavity, reasonable and competent provision of dental care. It is recommended to prescribe multivitamin complexes of vitamins A and E in the form of solutions taken orally and application. At the same time, the incidence of incurable diseases remains high, which requires the search for new treatment approaches based on fundamentally different mechanisms of action on pathological foci of leukoplakia.

One of the most relevant and effective methods of treating patients with precancerous diseases of the oral cavity is photodynamic therapy (PDT), which is a method of local activation of a special drug, a photosensitizer (PS), selectively accumulated in pathologically altered tissue, with visible red light. Subsequently, in the presence of tissue oxygen, photochemical reactions are initiated with the formation of reactive oxygen species (singlet oxygen, superoxide anion, etc.), initiating oxidative stress syndrome in tissues that have accumulated PS, and their subsequent death through apoptosis, necrosis, autophagy and paraptosis depending on a number of factors [9, 10, 11]. In the case of PS accumulation in the endothelium of blood vessels, subsequent exposure to laser radiation leads to the formation of blood clots, vasoconstriction, vascular stasis and, consequently, the development of ischemic necrosis [12, 13]. An important role in tissue photodamage is played by the activation of the immune system, which manifests itself in the infiltration of pathologically altered tissues by neutrophils and macrophages in response to irradiation and is accompanied by the release of cytokines and inflammatory mediators. Activation of T lymphocytes leads to the destruction of the remaining pathologically altered cells [12, 13].

In recent years, the world has gained experience in using PDT with application and injection forms of various PS (5-aminolevulinic acid (5-ALA), chlorins, etc.) for patients with leukoplakia SOP. The results of both pilot studies on small samples of patients and systematic reviews of the literature involving hundreds of patients have been published. The use of PDT shows good tolerability, fairly high rates of complete regressions (from 22.58% to 100%) with a relapse rate (from 0% to 60%) [14, 15, 16].

All of the above makes further research in the field of application of laser technologies in the treatment of precancerous diseases of the oral mucosa relevant and promising.

The purpose of this research was to study the tolerability and effectiveness of the PDT method with chlorine PS photolon for patients with leukoplakia of the oral mucosa.

Materials and Methods

Patients

The retrospective study included 223 patients with a morphologically verified diagnosis of «Leukoplakia of the oral mucosa», who received treatment at the department of hyperthermia and photodynamic therapy (N.N. Alexandrov National Cancer Center pf Belarus, Republic of Belarus) from 2013 to 2023. According to clinical forms, the flat form predominated – 211 (94.6%) patients; verrucous form – 12 (5.4%) patients. Men made up 151 observations (67.7%) of the total cohort, women – 72 (32.3%). Age ranged from 28 to 76 years, average – 51.9±8.9 years.

The criteria for inclusion of patients in the study were:

- age 18 or more;
- clinically and morphologically verified diagnosis of «leukoplakia»;
- informed consent of the patient to undergo treatment using the PDT method.

Exclusion criteria were:

- morphologically verified cancer of the oral cavity;
- presence of contraindications for intravenous administration of PS;
- absence of severe concomitant pathology of the cardiovascular system, liver and kidneys in the stage of decompensation;
- patient's refusal to undergo PDT treatment.

The diagnosis was established on the basis of clinical and instrumental examination and morphological verification. When verifying the process, cytological or histological examination of pathologically altered tissues was used.

Ethical aspects

The study was designed in accordance with the Declaration of Helsinki (1975, revised 64th WMA General Assembly, Fortaleza, Brazil, 2013). All patients were informed about the PDT method used by the PS, possible adverse reactions, the need to adhere to the light regime in the first 3-4 days after treatment, the timing of follow-up visits for recovery, alternative treatment options, and gave written consent to the use of this treatment method.

Taking into account the fact that the treatment of patients with leukoplakia of the oral mucosa is not included in the National standards for the treatment of this pathology in the Republic of Belarus and in section 4.1 «Indications for use» of the General characteristics of the drug photolon (Order of the Ministry of Health of the Republic of Belarus dated November 28, 2022 № 1659), inclusion of patients in this study was carried out on the basis of the Instructions for Use «Photodynamic (fluorescent) diagnosis and therapy of cancer and precancerous diseases of the mucous membrane of the oropharyngeal zone» (Developer institution – N.N. Alexandrov National Cancer Center of Belarus, authors: Istomin Yu.P., Chalov V.N., Yaskevich L.S., Neiman O.I.), approved by the Ministry of Health of the Republic of Belarus, dated December 23, 2011, № 112-1111).

Photosensitizer

In the study, chlorin e6 was used as a pharmaceutical substance produced (RUE «Belmedpreparaty», Republic of Belarus, registration certificate № 16/11/886 dated November 8, 2016), which is a complex of trisodium salt of chlorine e6 with povidone with a K-value of 17. The drug diluted in 200 ml of 0.9% sodium chloride solution and administered intravenously over 30 minutes at a dosage of 1.7 to 2.5 mg/kg body weight, in a darkened room.

Photodynamic therapy

Before and after every PDT session, the radiation power at the output of the light guide was controlled using a production device («Solar», Republic of Belarus). The PDT session was carried out 2-2.5 hours after the end of the PS infusion using a semiconductor laser device «UPL PDT», produced («Lemt BelOMO», Republic of Belarus) with $\lambda = 665\pm5$ nm. To supply radiation, a fiber-optic light guide with a lens diffuser for external irradiation was used. The laser radiation dose density varied from 25 to 100 J/cm^2, the laser radiation power density varied from 0.07 to 0.32 W/cm^2, and the duration of one irradiation field varied from 2 to 13.5 minutes, depending on its linear dimensions. The number of irradiation sessions ranged from 1 to 6 depending on the area of pathological foci.

Depending on the location, area and clinical form of the disease, two types of radiation were used: in the first case, if the size of the pathological focus did not exceed 1 cm, the distal end of the light guide was brought into direct contact with the surface of the oral cavity; in the second case, if the size of the pathological lesion exceeded 1 cm, the radiation was applied remotely, perpendicular to its surface, and, if necessary, the entire surface of the formation was irradiated with several fields. The irradiation zone included pathologically altered tissues and an area of visually normal tissues at a distance of at

least 3-5 mm from the border of the pathology. Screening of surrounding intact tissue was not performed [17]. For pain relief, 15-20 minutes before the start of treatment, all patients were intramuscularly injected with ketorolac (4 ml); in some cases, local anesthesia was performed with lidocaine 2% in the form of a spray – 2-5 ml [3, 18].

Tolerability of treatment

The frequency and severity of adverse events when using PDT were assessed within 1-30 days after treatment using the CTCAE scale (version 4.03, dated 2010).

Treatment effectiveness

Control examinations were carried out after 7 days; 1, 3 and 6 months after treatment. The immediate results of treatment of patients with leukoplakia of the oral mucosa were assessed based on WHO criteria [3]:

- complete regression (CR) – complete disappearance of all manifestations of the disease, established both visually and by palpation and confirmed by negative results of a morphological study within 1-3 months after treatment;
- partial regression (PR) – a decrease in the pathological focus (or formations) by 50% or more, or when, in the clinically complete absence of pathology, tumor cells are revealed during a morphological study;
- tumor reduction by less than 50% or no change in tumor size was regarded as no effect (NE).

Results

No serious adverse reactions (anaphylactic shock, bronchospasm, drop in blood pressure, etc.) corresponding to CTCAE (grades III-IV) associated with PS infusion were recorded. There were also no symptoms of cutaneous phototoxicity (skin itching, hyperemia, conjunctivitis). In isolated cases, in the early post-procedural period (1-5 days after the administration of PS and the PDT session) a low-grade fever (+37.0-37.7°C) was noted, which corresponds to CTCAE (grades I-II). On days 1-3 after treatment, the formation of photo-induced hemorrhagic necrosis with exudation and swelling of surrounding tissues, followed by the formation of fibrinous deposits, was noted at the irradiation site. In a number of observations, patients experienced swelling of the soft tissues of the face of varying severity, which disappeared on its own after 1-5 days.

Within 2-8 days after treatment, patients noted moderate pain in the affected area, which was well relieved by the administration of non-narcotic analgesics and sedatives (CTCAE, grade I-II).

During control examinations up to 3 months after irradiation, the frequency of complete regressions in the general cohort of patients was 95.4% of observations (213 patients), partial regressions – 3.4% of observations (8 patients). An objective response rate of 98.8% was observed. In 1.2% of observations (2 patients), there was no effect on the treatment.

The distribution of PDT effects depending on the clinical form of the disease is presented in Table 1.

The period of observation of patients varies from 3 to 120 months (average 66 months). The frequency of disease relapses during this period was 9%.

Patients with partial regression and identified relapse of the disease were treated with repeated PDT sessions.

In connection with the development of necrosis of irradiated tissues, in order to prevent purulent-septic complications, it is necessary to constantly treat the COP with solutions of antiseptic drugs (furacilin, anti-inflammatory herbal preparations, etc.). Starting from 3-4 days after the PDT session, patients underwent applications with solcoseryl, Metrogyl Denta gel, and sea buckthorn oil in order to stimulate regenerative processes. Final epithelization of wound defects occurred, on average, 3-6 weeks after photoirradiation. In the overwhelming majority of observations, good

Таблица 1
Эффективность ФДТ с хлорином е6 у больных с различными клиническими формами лейкоплакии слизистой оболочки полости рта

Table 1
Efficacy of PDT with chlorin e6 in patients with various clinical forms of leukoplakia of the oral mucosa

Клиническая форма лейкоплакии Clinical form of leukoplakia	Полная регрессия, число пациентов и % Full regression, number of patients and %		Частичная регрессия, число пациентов и % Partial regression, number of patients and %		Отсутствие эффекта, число пациентов и % No effect, number of patients and %		Объективный ответ*, число пациентов и % Objective answer*, number of patients and %	
Плоская Flat	205	97,1	6	2,9	0	0	211	100
Веррукозная Verrucous	7	58,3	3	25	2	16,7	10	83,3

*Объективный ответ включает полные и частичные регрессии заболевания
*Objective response includes complete and partial regression of the disease

cosmetic results were achieved: the wound surface was epithelialized with minimal formation of scar tissue.

The results obtained in the study are illustrated by the following clinical examples (Fig. 1, 2, 3, 4 and 5).

Discussion

Leukoplakia of the oral mucosa is a precancerous disease, the treatment of which directly depends on a number of factors: the clinical form of the disease, the area and extent of pathologically altered tissues, the nature of previous treatment. In the treatment of a flat form, a conservative approach is relevant, namely, sanitation of the oral cavity, rational and competent provision of dental care. It is recommended to prescribe multivitamin complexes of vitamins A and E in the form of solutions taken orally and application. In the case of verrucous and erosive-ulcerative forms, surgical tactics predominate: pathologically altered foci are removed

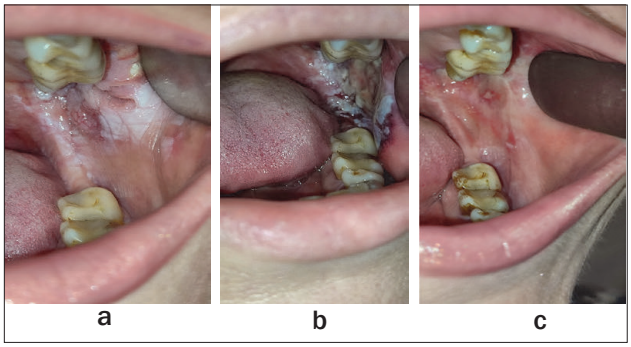

Рис. 3. Лейкоплакия щеки, плоская форма
a – состояние до ФДТ;
b – локальный статус через 3 сут после ФДТ (экспозиционная доза – 100 Дж/см^2);
c – локальный статус через 3 мес после ФДТ (достигнута полная регрессия)
Fig. 3. Leukoplakia cheeks, flat shape
a – state before PDT;
b – local status 3 days after PDT (exposure dose – 100 J/cm^2);
c – local status 3 months after PDT (full regression achieved).

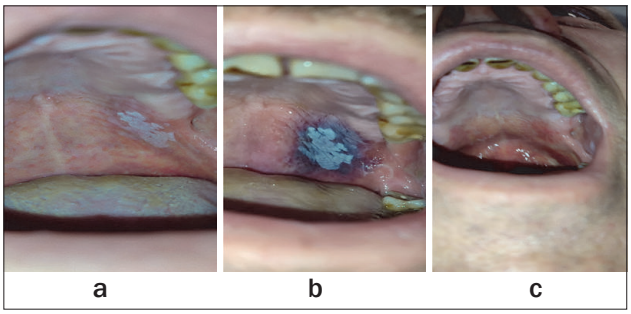

Рис. 1. Лейкоплакия мягкого неба, плоская форма
a – состояние до ФДТ;
b – локальный статус через 7 сут после ФДТ (экспозиционная доза – 100 Дж/см^2);
c – локальный статус через 3 мес после ФДТ (достигнута полная регрессия).
Fig. 1. Leukoplakia of the soft palate, flat form
a – state before PDT;
b – local status 7 days after PDT (exposure dose – 100 J/cm^2);
c – local status 3 months after PDT (complete regression achieved).

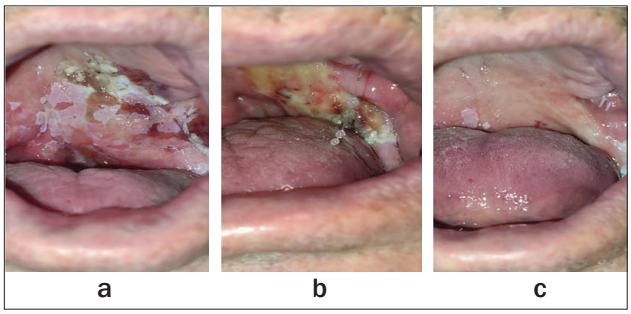

Рис. 4. Лейкоплакия мягкого неба, плоская форма
a – состояние до ФДТ;
b – локальный статус через 7 сут после ФДТ (экспозиционная доза – 100 Дж/см^2);
c – локальный статус через 3 мес после ФДТ (достигнута частичная регрессия; пациент продолжает лечение методом ФДТ).
Fig. 4. Leukoplakia of the soft palate, flat form
a – state before PDT;
b – local status 7 days after PDT (exposure dose – 100 J/cm^2);
c – local status 3 months after PDT (partial regression has been achieved; the patient continues treatment with PDT).

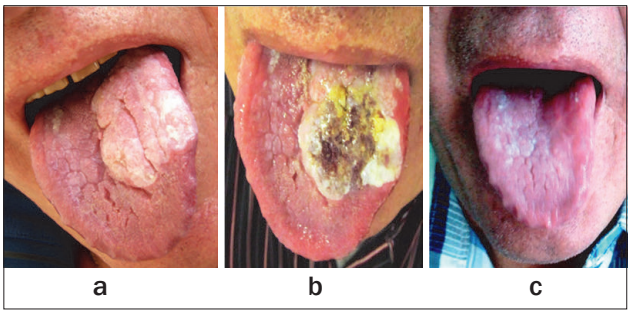

Рис. 2. Лейкоплакия тела языка, веррукозная форма
a – состояние до ФДТ;
b – локальный статус через 7 сут после ФДТ (экспозиционная доза – 100 Дж/см^2);
c – локальный статус через 3 мес после 3 сеансов ФДТ (достигнута полная регрессия)
Fig. 2. Leukoplakia of the body of the tongue, verrucous form
a – state before PDT;
b – local status 7 days after PDT (exposure dose – 100 J/cm^2);
c – local status 3 months after 3 PDT sessions (full regression achieved).

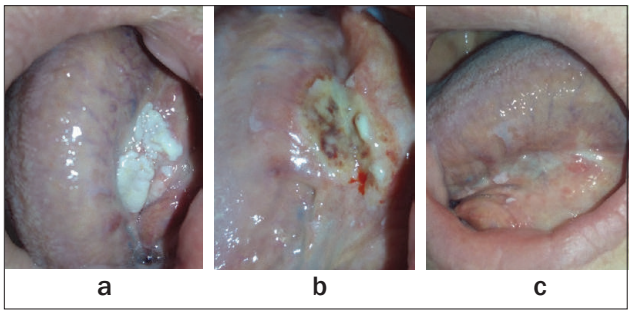

Рис. 5. Лейкоплакия языка, плоская форма
a – состояние до ФДТ;
b – локальный статус через 5 сут после ФДТ (экспозиционная доза – 100 Дж/см^2);
c – локальный статус через 3 мес после ФДТ (достигнута полная регрессия).
Fig. 5. Leukoplakia of the tongue, flat shape
a – state before PDT;
b – local status 5 days after PDT (exposure dose – 100 J/cm^2);
c – local status 3 months after PDT (complete regression achieved).

using laser Nd:YAG or CO_2 ablation of tissues affected by leukoplakia. Despite the variety of ways to treat this disease, the incidence of recurrence of the disease remains high. For example, in a study by J.M. White et al. (1998) in a comparative aspect, the long-term results of the use of Nd:YAG and CO2 in the treatment of 39 patients with oral leukoplakia were studied. According to the authors, the recurrence rate of the disease with the use of these therapeutic options was 27% and 24%, respectively [19]. W. Gushiken de Campos et al. (2022) presented the results of the use of CO_2 laser therapy in 37 patients with oral leukoplakia, who had an average follow-up period of 38.2 months in 35.1% of cases with a recurrence of the disease [20]. The presumed reasons for the high percentage of recurrences in patients with this pathology using conservative approaches, surgical tactics and laser methods are exclusively mechanical impact on leukoplakia, multiple nature of the lesion and possible presence of clinically undetectable foci of the disease. All of the above requires the search for new approaches to treatment based on fundamentally different mechanisms of influence on pathological foci of leukoplakia of the oral mucosa.

One of these methods is the PDT method, which has proven to be a well-tolerated and effective option for the therapeutic treatment of precancerous diseases of the oral mucosa. The effectiveness of the method directly depends on the clinical form of the disease, the nature of the lesion (single or multiple foci), previous treatment, etc. In our retrospective study based on the analysis of the treatment results of 223 patients with primary oral mucosal leukoplakia, the frequency of complete and partial regressions for flat and verrucose forms was 97.1% and 58.3%, as well as 2.9% and 25%, respectively. The recurrence rate of the disease for the follow-up period from 3 to 120 months was 9%. In our opinion, the different efficacy of PDT in patients with flat and verrucous forms of leukoplakia is associated with both the peculiarities of PS accumulation in pathologically altered tissues (a high concentration of the drug in the flat form) and, potentially, a more pronounced exophytic component in the verrucous form, which does not allow laser radiation to penetrate sufficiently to the entire depth of the focus leukoplakia. Thus, the depth of penetration of laser radiation with λ=660±5 nm, specific for chlorin e6, into biological tissues is on average 7 mm.

In recent years, the world has accumulated experience in the use of PDT with application and injection forms of various PS (5-ALA, chlorines, etc.) in several thousand patients with leukoplakia of the oral mucosa. Various teams of authors from large research centers and clinics in Europe, Southeast Asia, and the USA have published both the results of pilot studies on small samples of patients and systematic literature reviews involving hundreds of patients. In the publications presented below, a lower percentage of objective responses to treatment with PDT was primarily associated with the use of application forms of PS (5-ALA), the local application of which to pathological foci did not allow achieving a higher concentration of PS compared to intravenous administration, which affected the effectiveness of PDT. It is also worth mentioning the fact that the distribution of PS, as a rule, is not homogeneous during application due to the anatomical features of the structure of the oral mucosa. This is due to the presence of crypts, folds, various vascularization, keratinization of the epithelium or its absence. And, of course, an important aspect is λ=630 nm, which makes it possible to activate 5-ALA, which has accumulated in pathologically altered tissues. In this case, active photodynamic exposure is possible to a depth of 1 to 1.5-2 mm, which significantly reduces the therapeutic possibilities of PDT with this PS.

In the case of photofrin II or its analogues (λ=629-635 nm), this indicator is, on average, 2-3 mm. These PS are used for PDT for oral mucosal leukoplakia in injectable forms. Nevertheless, it is the insignificant depth of effective photodynamic impact that leads, in some cases, to an increase in the frequency of local recurrences of the disease and partial regressions.

Selected results of the use of PDT in the treatment of precancerous diseases of the oral mucosa with various PS, published as part of systematic reviews of the literature, are presented in Table 2.

As the results of numerous foreign studies show, the PDT method is an effective option for organ-preserving treatment of patients with leukoplakia of the oral mucosa, which is confirmed by a fairly high frequency of objective responses (according to various data, from 22.58% to 100%) to the treatment [14, 15, 16, 21, 22]. However, despite the optimistic immediate the frequency of registered recurrences of the disease remains high (up to 60%), which requires the search for new and further optimization of existing treatment regimens for this category of patients using the most effective PDT parameters. For this purpose, it is advisable to conduct multicenter randomized clinical trials on large samples of patients.

Conclusion

It is a well-known fact that the main advantages of PDT in comparison with traditional methods of treatment of precancerous diseases of the oral mucosa are minimal toxicity to normal tissues surrounding pathological foci due to the selective accumulation of PS in the tissues of leukoplakia, a low risk of serious adverse reactions, the possibility of an outpatient session, the possibility of repeated treatment in case of large area of lesion, good cosmetic results [23, 24]. These positive aspects of PDT make it possible not only to ensure the effective use of the method, but also to significantly improve the quality of life of the treated patients.

Таблица 2
Эффективность ФДТ у больных с предраковыми заболеваниями слизистой оболочки полости рта
Table 2
The effectiveness of PDT in patients with precancerous diseases of the oral mucosa

Авторы, год, страна, ссылка Authors, year, country, link	Число исследований, число пациентов Number of studies, number of patients	Клинические диагнозы Clinical diagnosis	ФС PS	Параметры ФДТ PDT parameters	Частота ПР, ЧР и ОЭ, % Frequency of CR, PR and NR, %	Частота рецидивов, % Relapse rate, %
Vohra F., 2015, США, [14] Vohra F., 2015, USA, [14]	13 5-147 13 5-147	Лейкоплакия, эритроплакия, эритро-лейкоплакия, веррукозная гиперплазия Leukoplakia, erythroplakia, erythro-leukoplakia, verrucous hyperplasia	5-АЛК, хлорин е6, фоскан, фотофрин II 5-ALA, chlorine e6, foscan, photofrin II	λ=585-660 нм 0,1-0,15 Вт/см^2 1-16,5 мин. λ=585-660 nm 0.1-0.15 W/cm^2 1-16.5 min.	27-100 5-50 0-25 27-100 5-50 0-25	>36 >36
Gondivkar S.M., 2018, Индия, [15] Gondivkar S.M., 2018, India, [15]	26 2-147 26 2-147	Лейкоплакия, эритроплакия, эритро-лейкоплакия, веррукозная дисплазия Leukoplakia, erythroplakia, erythro-leukoplakia, verrucous dysplasia	5-АЛК, хлорин е6, фотосан, гемато-порфирин, фотофрин II 5-ALA, chlorine e6, photosan, hemato-porphirin, photofrin II	λ=585-652 нм 0,05-0,5 Вт/см^2 1-143 мин. λ=585-652 nm 0.05-0.5 W/cm^2 1-143 min.	22,58-100 4-66 0-38,7 22.58-100 4-66 0-38.7	- -
Li Y., 2019, Китай, [16] Li Y., 2019, China, [16]	16 352 16 352	Лейкоплакия, Дисплазия Leukoplakia, dysplasia	5-АЛК, метиленовый синий, хлорин е6 5-ALA, methylene blue, chlorine e6	λ=420-660 нм 0,1-0,15 Вт/см^2 1-16,5 мин. λ=420-660 nm 0.1-0.15 W/cm^2 1-16.5 min.	32,9 43,2 - 32.9 43.2 -	0-60 0-60

*ФС – фотосенсибилизатор, ФДТ – фотодинамическая терапия, ПР – полная регрессия, ЧР – частичная регрессия, ОЭ – отсутствие эффекта
*PS – photosensitizer, PDT – photodynamic therapy, CR – complete regression, PR – partial regression, NR – no response

As a result, summarizing our own data, demonstrating that the method is well tolerated (no serious adverse reactions of III-IV degree and minimal risk of developing stage I-II reactions according to the CTCAE classification), a high frequency of objective responses to the treatment (100% for the flat form and 83.3% for the verrucous form) and a low risk of disease recurrence (9%), PDT with chlorin e6 can be recommended as a highly effective alternative standard methods of treatment of patients with leukoplakia of the oral mucosa, as well as a way to prevent the development of malignant neoplasms of this localization.

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PHOTODYNAMIC THERAPY IN THE TREATMENT OF HPV-ASSOCIATED CERVICAL CANCER: MECHANISMS, CHALLENGES AND FUTURE PROSPECTS

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Abstract

Photodynamic therapy (PDT) has shown promise as a modality for the treatment of cervical cancer caused by the human papillomavirus (HPV). This review provides a comprehensive examination of the role of PDT in overcoming the challenges presented by conventional treatments for cervical cancer. Beginning with an overview of the relationship between cervical cancer and HPV infection, the review introduces the principles of PDT, its mechanism of action, and its potential as an innovative treatment strategy. The review highlights preclinical studies in animal models that demonstrate the efficacy of PDT in targeting HPV-infected cervical cells and provide mechanistic insights into its cytotoxic effects. We reviewed clinical studies and case reports highlighting the potential of PDT as an alternative or adjunctive treatment option. Challenges and limitations, including depth of light penetration, photosensitizer specificity, and standardization of protocols, will be discussed in the context of potential side effects and comparison with conventional treatments. Future directions include ongoing research, combination therapies with immunotherapy or targeted agents, advances in photosensitizer development, and personalized approaches. The advancement of PDT promises to change the landscape of HPV-associated cervical cancer treatment by providing a targeted, personalized, and minimally invasive approach.

Keywords: cervical cancer, human papillomavirus (HPV), photodynamic therapy (PDT), combination therapies, photosensitizers.

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ФОТОДИНАМИЧЕСКАЯ ТЕРАПИЯ В ЛЕЧЕНИИ ВПЧ-АССОЦИИРОВАННОГО РАКА ШЕЙКИ МАТКИ: МЕХАНИЗМЫ, ПРОБЛЕМЫ И ПЕРСПЕКТИВЫ НА БУДУЩЕЕ

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Резюме

Фотодинамическая терапия (ФДТ) зарекомендовала себя как многообещающий метод лечения рака шейки матки, вызванного вирусом папилломы человека (ВПЧ). В этом обзоре всесторонне рассматривается роль ФДТ в преодолении проблем, связанных с традиционными методами лечения рака шейки матки. Начиная с обзора взаимосвязи между раком шейки матки и ВПЧ-инфекцией, в обзоре представлены принципы ФДТ, механизм ее действия и ее потенциал в качестве инновационной стратегии лечения. В обзоре освещены доклинические исследования на животных моделях, которые демонстрируют эффективность ФДТ в отношении клеток шейки матки, инфицированных ВПЧ и дают представление о механизмах ее цитотоксического действия. Мы рассмотрели клинические исследования и отчеты о случаях, в которых подчеркивается потенциал ФДТ как альтернативного или дополнительного метода лечения. Проблемы и ограничения, включая глубину проникновения света, специфичность фотосенсибилизаторов и стандартизацию протоколов, будут обсуждаться в контексте потенциальных побочных эффектов и сравнения с традиционными методами лечения.

Будущие направления включают текущие исследования, комбинированную терапию с иммунотерапией или таргетными препаратами, достижения в разработке фотосенсибилизаторов и персонализированные подходы. Развитие ФДТ обещает изменить подход к лечению рака шейки матки, ассоциированного с ВПЧ, за счет обеспечения целенаправленного, персонализированного и минимально инвазивного подхода.

Ключевые слова: рак шейки матки, вирус папилломы человека (ВПЧ), фотодинамическая терапия (ФДТ), комбинированная терапия, фотосенсибилизаторы.

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Introduction

Cervical cancer remains a major global health problem and is the fourth most common cancer in women worldwide [1, 2, 3, 4]. The central role of persistent human papillomavirus (HPV) in the development of cervical cancer is now well established [5, 6]. HPV, especially high-risk genotypes such as HPV-16 and HPV-18, contribute to the malignant transformation of cervical epithelial cells, so there is an urgent need for innovative and effective treatment strategies [7, 8]. Among these strategies, photodynamic therapy (PDT) is a promising approach [9].

Originally developed at the beginning of the 20th century, PDT has evolved into a new, minimally invasive treatment method in various medical disciplines. PDT involves the introduction of a photosensitizing agent that accumulates primarily in malignant tissue, followed by local activation by light of a specific wavelength [10, 11]. This activation leads to reactive oxygen species (ROS) forming, which causes cytotoxic effects that attack and destroy malignant cells [12, 13]. The non-invasive nature of PDT, selective tissue targeting, and potentially minimal systemic toxicity make it an attractive approach for diseases such as cancer. Some authors have questioned the radical nature of PDT in cancer, pointing to the impossibility of penetrating a beam of light at a distance (up to 1 cm) with a progressive loss of radiant power [14, 15]. In contrast, Chizenga E.P. et al. have asserted the widespread introduction of PDT for non-invasive and minimally invasive cancers of the cervix and cervical canal [16].

The purpose of this review is to provide a comprehensive assessment of the existing preclinical studies and clinical trials that have studied the use of PDT for cervical cancer associated with HPV, which may allow researchers and clinicians to determine its appropriate role in the treatment of this pathology and its place in the arsenal of therapeutic effects. By summarizing the available evidence, this review aims to clarify PDT's efficacy, safety profile, and potential benefits compared to traditional treatments. In addition, the mechanical aspects underlying the effects of PDT on HPV-infected cervical cells are considered, shedding light on its immunomodulatory effects and potential synergies with new immunotherapy methods.

In conclusion, this review aims to contribute to the evolving landscape of cervical cancer treatment by highlighting the untapped potential of PDT. By exploring the mechanisms, clinical outcomes, challenges, and future directions, we aim to provide clinicians, researchers, and policymakers with a comprehensive understanding of the role of PDT in the fight against HPV-associated cervical cancer and stimulate further research to optimize its therapeutic potential.

HPV-Associated cervical cancer: pathogenesis and current treatment approaches

The human papillomavirus (HPV) is a diverse group of DNA viruses, of which many genotypes are known to infect the genital mucosa [17, 18]. While most HPV infections are transient and benign, persistent infection with high-risk HPV genotypes such as HPV-16 and HPV-18 plays a central role in the development and progression of cervical cancer [19]. These oncogenic strains encode the viral oncoproteins E6 and E7, which inactivate the tumor suppressor proteins p53 and pRB, respectively, disrupting normal cell cycle regulation and promoting cell transformation [20, 21, 22].

The pathogenesis of HPV-associated cervical cancer is a multistep process involving the interaction of viral and host factors. Integration of the virus into the host genome leads to dysplastic changes in the cells of the cervical epithelium [23, 24]. Persistent infection promotes progression from low-grade cervical intraepithelial neoplasia (CIN) to highly differentiated CIN and finally to invasive carcinoma. Activation of oncogenic signaling pathways and evasion of immune surveillance contribute to tumor growth and metastasis [25].

Modern approaches to treatment of cervical cancer

1. Surgery: Surgery remains the cornerstone of cervical cancer treatment. Depending on the stage of the tumor, surgical options include hysterectomy,

radical hysterectomy, and lymphadenectomy. While surgical removal of the tumor and surrounding tissue can be curative in the early stages of the disease, it may not be sufficient in advanced stages with lymph node involvement or metastasis [26, 27].

2. Radiation therapy: Radiation therapy, often in combination with chemotherapy, is frequently used for locally advanced cervical cancer. External beam radiation and brachytherapy effectively target the tumor site and attempt to destroy the cancer cells while sparing nearby healthy tissue. The combination of radiation and concurrent chemotherapy improves outcomes by increasing the sensitivity of tumor cells to radiation-induced damage [27, 28].

3. Chemotherapy: Chemotherapy plays a critical role in the primary treatment of locally advanced cancer and in the adjuvant treatment of cervical cancer. Platinum-based therapies, such as cisplatin, are often used to increase the efficacy of radiotherapy. In addition, systemic chemotherapy can be used for metastatic or recurrent disease [29].

Although these treatment approaches are successful, they are not without limitations. Surgery is associated with the risk of postoperative complications, and radiation therapy can lead to long-term side effects such as rectovaginal fistula, obliteration of the cervical canal with a hematometer, and radiation castration [10, 30]. Chemotherapy is not very effective when used once and is associated with systemic toxicity [31]. In addition, factors such as tumor heterogeneity, resistance, and patient characteristics (age, comorbidities, etc.) may limit the therapeutic efficacy of these approaches.

Against the background of these problems, investigating innovative treatment strategies such as PDT becomes an urgent necessity. The selective effect of PDT, the possibility of reducing side effects, and the potential synergy with existing treatments are promising for improving the treatment of HPV-associated cervical cancer. By overcoming the limitations of existing treatments and offering new intervention options, PDT can help improve patient outcomes and quality of life.

Photodynamic Therapy (PDT): mechanism and principles

PDT is a state-of-the-art therapeutic approach that uses the power of light and photosensitizers to destroy malignant cells specifically [12]. The basic principle of PDT is the unique interaction of three key components: a photosensitizer, specific wavelengths of light, and molecular oxygen. When these components come together, they trigger a cascade of events that culminate in the selective destruction of tumor cells while sparing the surrounding healthy tissue [1, 12].

Photosensitizers play a central role in the efficacy of PDT. These molecules are usually non-toxic compounds that, when activated by light of specific wavelengths, transition from a ground state to an excited state with higher energy [32]. Photosensitizers can be divided into different classes, such as porphyrins, phthalocyanines, and chlorins, each with unique spectral properties. The choice of photosensitizer is critical because it determines the wavelengths of light that must be used for optimal activation [33]. The type of photosensitizer is controversial because laser devices are tied to the chemical structure, which depends on the absorption spectrum [12]. In Russia and China, chlorine and the red diode laser with 662 nm have traditionally dominated [12]. In Europe, porphyrins are used as photosensitizers, but it is impossible to evaluate their efficacy due to methodological differences [13, 14].

Light sources activate photosensitizers that emit wavelengths corresponding to the selected photosensitizer's absorption spectra. As a rule, lasers or light-emitting diodes (LEDs) are used [1]. The wavelength and light intensity are carefully selected according to the optimal absorption properties of the photosensitizer. This controlled activation initiates the energy transfer process, causing the photosensitizer to return to its ground state and simultaneously releasing energy in the form of reactive oxygen species (ROS) [12].

When the light is activated, the photosensitizers interact in their excited state with molecular oxygen in the surrounding tissues [16, 33]. This interaction leads to ROS, especially singlet oxygen ($^1O_2$), highly reactive and cytotoxic molecules [34]. These ROS trigger oxidative stress by damaging cellular components such as lipids, proteins, and DNA. In tumor cells, impaired antioxidant defense mechanisms make them more susceptible to ROS-mediated damage, leading to cell death by apoptosis, necrosis, or autophagy [35, 36, 37, 38].

One of the remarkable features of PDT is its selectivity: the photosensitizer accumulates primarily in the tumor tissue due to its increased permeability and retention. This enables targeted destruction of cancer cells with minimal damage to normal tissue, thus reducing systemic toxicity and side effects associated with the treatment. PDT's dual mechanism of action, which involves direct damage to the cells and activation of the immune system, further enhances its potential as an effective therapeutic method [10].

The extraordinary precision of the molecular interactions of PDT and the synergistic effect of light, photosensitizer, and oxygen opens up excellent prospects for treating HPV-associated cervical cancer. By exploiting innate biochemical differences between normal and malignant cells, PDT offers an innovative strategy that meets the requirements for personalized, minimally invasive treatments in oncology [9].

Preclinical studies on PDT for HPV-associated cervical cancer

Before introducing a therapeutic approach into clinical practice, thorough preclinical studies must assess safety, efficacy, and understanding of mechanisms. In the context of PDT for HPV-associated cervical cancer, animal models have served as a tool to evaluate the potential of this treatment modality. These models, often using rodents such as mice or rabbits, provide researchers with a controlled environment to simulate various aspects of human cervical cancer and to study the effects of PDT in a controlled and systematic manner [39, 40].

Preclinical studies in animal models have consistently demonstrated the efficacy of PDT in HPV-infected cervical cells. These studies usually involve inoculation of animals with HPV-positive cervical tumor cells and subsequent treatment with photosensitizers and light exposure [41]. The selectivity of PDT is apparent, as the photosensitizer accumulates mainly in the tumor tissue due to its inherent properties, leading to the destruction of the malignant cells when activated by light. In subsequent genetic studies on these animals, no HPV DNA could be detected after 3, 6, and 12 months [22, 23, 24].

These studies often include the evaluation of tumor regression, tumor size reduction, and tumor growth inhibition. In addition, they provide information on the effects of treatment on various biological parameters, such as impairment of vascular function in the tumors, immune responses, and potential tumor recurrence. Such information is invaluable for understanding the broader impact of PDT in the context of HPV-associated cervical cancer [16, 42, 33].

Preclinical studies confirm the efficacy of PDT and provide a mechanistic understanding of how this treatment method exerts its effects. These studies focus on the molecular and cellular mechanisms underlying the cytotoxic effects of the reactive oxygen species (ROS) generated during PDT [34, 36, 12].

Mechanistic studies frequently show that ROS-induced oxidative stress triggers cellular responses, including DNA damage, activation of apoptotic signaling pathways, and modulation of immunomodulatory signals. In addition, the effect of PDT on the tumor microenvironment, such as tumor vascularization and immune cell infiltration, contributes to the overall development of the treatment [35, 36, 37, 38].

In addition, animal models allow researchers to study parameters critical for the optimization of PDT, such as the ideal dose of photosensitizer, light intensity, and the interval between photosensitizer administration and light exposure. These parameters significantly impact treatment outcomes, and preclinical studies have helped establish recommendations for proper calibration [41].

In summary, preclinical studies in animal models serve as a link between basic research and the clinical application of PDT for HPV-associated cervical cancer. These studies not only confirm the efficacy of PDT in targeting HPV-infected cervical cells but also provide a deeper understanding of the mechanisms underlying this treatment. The information obtained from animal models is used in the design of clinical trials and serves as a guide to make PDT a safe and effective therapeutic option for patients [34, 35].

Clinical studies and case reports

The transition from preclinical research to clinical practice is an essential step in evaluating the potential of PDT as a treatment for HPV-associated cervical cancer.

The first attempts to use PDT to treat precancerous lesions and early cervical cancer were made in the early 1980s [1]. This attempt was made regardless of whether the women were infected with HPV. Once the etiologic role of the virus in the development of intraepithelial neoplasia and cervical cancer had been confirmed [28, 23, 24], the focus shifted to the highly oncogenic HPV 16 and 18 PDT.

Clinical trials provide information on PDT's safety, efficacy, and tolerability under real-life conditions. These studies have several objectives, including evaluating treatment outcomes, optimizing PDT protocols, and comparing PDT with traditional treatment approaches [12, 13].

Clinical trials have shown promising treatment outcomes and response rates in patients with HPV-associated cervical cancer who have undergone PDT [14, 15]. Parameters such as tumor regression, lesion size reduction, and patient survival are usually evaluated. Some studies have shown that PDT can effectively induce tumor necrosis and lead to complete or partial remission, especially at an early stage of the disease [16]. In addition, the ability of PDT to preserve fertility and anatomical integrity in small tumor spreads is critical.

Comparative studies between PDT and traditional treatments provide valuable information on the potential of PDT as an alternative and/or adjunctive option [34, 39]. These comparisons often include an assessment of treatment efficacy, quality of life, and adverse events [18, 17]. Although PDT's non-invasive nature and targeted approach are advantageous, its effectiveness depends on tumor stage, size, and location factors. A comparative analysis helps clinicians decide the most appropriate treatment strategy based on the individual patient profile.

Including case reports in clinical studies emphasizes the practical application of PDT and its effects on individual patients. These reports include detailed descriptions of patient histories, treatment protocols, and post-treatment outcomes [22, 33, 30]. The reports of successful PDT interventions demonstrate the potential of the treatment to achieve favorable results even in complex cases. In addition, the case reports shed light on factors contributing to PDT's success, such as proper

patient selection, optimal dosage of photosensitizer, and individualized light delivery strategies.

These case reports also illuminate the patient experience and address treatment tolerance, recovery time, and long-term effects. They contribute to a more comprehensive understanding of the feasibility of PDT and patient satisfaction and provide a more complete picture of the clinical benefits of treatment [12].

Clinical studies and case reports, therefore, play a key role in bridging the gap between theoretical efficacy and actual applicability of PDT for HPV-associated cervical cancer. These studies demonstrate the potential of PDT as an innovative and targeted therapeutic approach and provide insights into treatment outcomes, comparative analysis, and individual patient progression. As clinical research in this area continues to evolve, these findings will help to improve PDT protocols and expand its role in the complex management of cervical cancer [12, 13, 42].

In the Russian Federation, the PDT method has found its place in treating several localizations at the level of approved federal clinical recommendations in its form or as part of complex therapy [43-46]. There are no similar recommendations for HPV-associated neoplasia. There is a collection of information on the effect of PDT on viral transmission and the duration of elimination of oncogenic HPV types [12].

Challenges and limitations

Despite its promising potential, the use of PDT in the treatment of cervical cancer is fraught with difficulties. These problems must be solved to fully exploit PDT's benefits in this context.

1. Depth of light penetration: One of the main problems is the limited penetration depth of the light into the tissue. Cervical cancer is often characterized by variable lesion depth, and it can be challenging to ensure adequate light delivery to deep-seated tumors. This limitation can lead to inconsistent treatment efficacy and incomplete tumor ablation [19, 20].
2. Photosensitizer specificity and tumor targeting: Choosing the right photosensitizer and achieving optimal tumor targeting are critical for the success of PDT. Photosensitizers should primarily accumulate in the tumor tissue, minimize uptake into healthy tissue, and minimize potential damage to surrounding structures [41].
3. Standardization of PDT protocols: The lack of standardized protocols for PDT is a serious problem. Variables such as photosensitizer dose, light intensity, and time between photosensitizer administration and light exposure can significantly affect treatment outcomes. Standardization is critical to ensure reproducibility and comparability between studies and clinical settings [32, 33].

Like any medical procedure, PDT is associated with potential side effects and adverse events. After PDT treatment, photosensitivity reactions may occur, characterized by skin photosensitivity (photodermatoses). Other potential side effects include local inflammation, pain, and swelling at the treatment site. Adequate patient education and post-treatment care are essential to control and reduce these effects [11, 34, 35, 47].

It is essential to consider the limitations of PDT in the context of a broader range of treatment options for cervical cancer. Surgery, radiation therapy, and chemotherapy also have their limitations, including potential complications, systemic toxicity, and problems in treating advanced stages. Comparing the limitations of PDT with those of other treatment modalities helps clinicians and researchers make informed decisions about treatment choices based on individual patient characteristics and disease stage [17, 18, 26, 48].

Despite these challenges, ongoing research and innovation in PDT address many limitations. Advances in the development of photosensitizers, methods of light delivery, and combination therapy with immunomodulatory agents are gradually overcoming the obstacles and expanding the clinical use of PDT to treat cervical cancer [27, 28, 29, 30].

In conclusion, recognizing and addressing the problems and limitations of PDT in the treatment of HPV-associated cervical cancer is critical to optimizing its clinical application. If these obstacles are overcome through innovative strategies, collaboration between interdisciplinary teams, and continued research, PDT can become a more effective and well-integrated component of a holistic approach to cervical cancer treatment.

Future directions and emerging srategies

The evolving landscape of PDT for cervical cancer is characterized by constant research and innovation aimed at improving and expanding its potential. Researchers are actively exploring new approaches to solve PDT-related problems, including improving light penetration, optimizing photosensitizer delivery, and improving treatment monitoring [31, 32]. Advanced imaging techniques, such as fluorescence-guided surgery, are being investigated to identify lesions and accurately guide PDT interventions [33].

Combination therapy is a promising approach for the future treatment of cervical cancer [64]. The ability of PDT to induce immunogenic cell death fits well with the new immunotherapy strategies. Combining PDT with immune checkpoint inhibitors or adoptive T-cell therapy can enhance the antitumor immune response and thus improve therapeutic outcomes. In addition, combining PDT with targeted agents that specifically modulate

tumor microenvironment factors can improve treatment effects and overcome the limitations of PDT [35, 36].

The development of photosensitizers is an active area of research to improve the efficacy of PDT. Researchers are developing photosensitizers with improved optical properties, increased selectivity for tumor cells, and reduced toxicity. In addition, targeted delivery systems such as nanoparticles or antibodies conjugated with photosensitizers are being developed to increase the accumulation of photosensitizers in tumors to improve targeting and therapeutic outcomes [37].

With the increasing importance of precision medicine, personalized approaches for PDT are also emerging. Tailoring PDT protocols based on specific patient characteristics, such as tumor biology, microenvironment, and genetic profiles, can optimize treatment outcomes. The use of advanced imaging and diagnostic methods to assess tumor characteristics in real time enables on-the-fly adjustment of the treatment plan, maximizing the efficacy of PDT while minimizing side effects [38].

In addition, predictive biomarkers are being identified to help select patients and predict response to treatment. Such an individualized approach not only increases the efficacy of treatment but also helps to reduce the number of patients and improve their quality of life.

The future of PDT in the treatment of HPV-associated cervical cancer is, therefore, characterized by a dynamic interplay of research and innovation. New strategies include an interdisciplinary approach involving oncologists, immunologists, material scientists, and imaging experts. As these advances come together, the landscape of cervical cancer treatment is likely to change, ushering in a new era of personalized, targeted, and minimally invasive therapies that can significantly improve patient outcomes and overall well-being [29].

Conclusion

This review comprehensively examined the role of PDT in the treatment of HPV-associated cervical cancer, leading to several important conclusions. The review included an in-depth understanding of the association of cervical cancer with HPV, the underlying principles of PDT, preclinical and clinical studies, issues, and directions for the future. The combination of the evidence and the results of the analysis allowed us to gain a holistic view of the potential of PDT to revolutionize the treatment of cervical cancer.

PDT is becoming increasingly popular in modern medicine. With a history of more than six thousand years and a Nobel Prize 120 years ago, PDT has only found widespread application in the last few decades. The dynamics of published papers show that PDT has become increasingly in demand in recent years and has found its way into various areas of modern medicine [40].

The potential of PDT is truly enormous: in the early stages of malignant neoplasms, it can be used as an alternative to radical surgical treatment and radiation treatment and in the advanced stages of cancer - as an adjunct to ongoing complex treatment. In progressive, non-responsive, and exhaustive options of traditional treatment methods, PDT is the only method that improves the quality of life by exerting local control [32, 42].

In conclusion, this review highlights the transformative potential of PDT in the treatment of HPV-associated cervical cancer. This review enhances the ongoing discussion on innovative therapeutic strategies by providing a concise overview of the major findings, validating its potential, and advocating further research and clinical trials. With continued commitment and interdisciplinary collaboration, PDT promises to change the landscape of cervical cancer treatment and offer new hope to patients and clinicians.

Implications for clinical practice

The integration of PDT into the treatment paradigm for HPV-associated cervical cancer requires a careful and strategic approach. Although PDT shows promise, its implementation requires considerations consistent with established clinical practice. PDT should be considered an additional or alternative therapeutic option that complements existing treatment modalities [34, 23].

Clinicians should evaluate the stage, size, and location of the cervical tumor and the patient's overall health status to determine the appropriateness of PDT. Collaboration with multidisciplinary teams is essential to develop comprehensive treatment plans that consider the benefits and limitations of PDT in the context of each patient's unique condition.

Patient selection is critical to the success of PDT. Ideal candidates for PDT are patients with localized early-stage cervical cancer who can benefit from targeted and minimally invasive treatment. Patients should be screened to determine whether they can tolerate light exposure and potentially develop a photosensitivity reaction [38, 42].

In addition, the criteria for patient selection should consider factors such as tumor histology, HPV genotype, and previous treatment history. Collaborative decision-making among medical oncologists, radiation oncologists, and PDT specialists can help identify patients who could benefit most from PDT while ensuring that it meets their individual preferences and goals.

Multidisciplinary tumor management boards can serve as platforms for collaborative decision-making, where treatment options are discussed, taking into account each patient's clinical, pathological, and radiological data. Such a collaborative approach ensures effective integration of PDT and optimizes treatment outcomes while minimizing risks [41, 42, 46].

Regular communication between these specialists is essential to improve treatment protocols, solve problems, and exchange opinions from a clinical and scientific perspective. As the field of PDT continues to evolve, ongoing collaboration ensures that clinical practice is in line with the latest evidence and innovations [41, 42, 46].

In conclusion, the incorporation of PDT into the clinical management of HPV-associated cervical cancer requires careful consideration, patient selection, and interdisciplinary collaboration. By drawing on the experience of oncologists, researchers, and PDT specialists, clinicians can realize the full potential of PDT and ensure its safe and effective integration into a broader treatment paradigm.

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IN MEMORY OF OSKAR IOSIFOVICH KOIFMAN

On December 31, 2023, Oskar Iosifovich Koifman, a scientist whose name is inscribed in golden letters in the history of Russian science and Ivanovo State University of Chemical Technology (ISCTU), passed away.

O.I. Koifman – Doctor of Chemical Sciences, Academician of the Russian Academy of Sciences, Honored Scientist of the Russian Federation, laureate of the Prizes of the Government of the Russian Federation in the field of science and technology and the President of the Russian Federation in the field of education. He was one of the leading scientists in the field of chemistry of tetrapyrrole macroheterocyclic compounds: porphyrins and their structural analogues, polymers based on them, as well as technology for the production and modification of synthetic and natural macroheterocycles and their practical use.

O.I. Koifman's scientific interests throughout his scientific career were related to the synthetic organic, physical, coordination, medical and applied chemistry of porphyrins, phthalocyanines, porphyrazines and their metal complexes, as well as supramolecular liquid crystals. Having united a young close-knit team around himself, he created a new scientific direction – the chemistry of macroheterocyclic compounds and porphyrin polymers.

A team of scientists led by O.I. Koifman has gained recognition in Russia and abroad. The leading scientific school headed by him is a multiple winner of the competition for the right to receive grants from the President of the Russian Federation for state support of leading scientific schools in the field of knowledge «Chemistry, New Materials and Chemical Technologies».

O.I. Koifman is the author of more than 1800 scientific and scientific-pedagogical works, including 11 monographs and 22 chapters in collective monographs, 87 copyright certificates and patents. Under his leadership, 28 candidates and 9 doctors of chemical sciences were trained.

O.I. Koifman is a member of the Presidium of the Educational Institutions of Chemical Technology, Head of the Department of Chemical Technology of the Academy of Sciences of the Russian Federation, a member of the Presidium of the Mendeleev Russian Chemical Society, the organizer of a large number of all-Russian and international conferences, as well as the only scientific and practical seminar in Russia for the exchange of experience between journals in the field of chemistry and chemical technology.

Oskar Iosifovich was awarded the Order of Honor of the Russian Federation, the Certificate of Honor of the President of the Russian Federation, the badge «Honorary Worker of Higher Education of Russia», the badge «Honorary Worker of Science and Technology of the Russian Federation», the Badge of Honor of the Mendeleev Russian Chemical Society, the medals named after N.N. Semenov and A.M. Prokhorov of the Prokhorov Academy of Engineering Sciences for outstanding achievements in the field of engineering sciences. He was awarded the title of «Honorary Chemist». In 2018, he became the winner of the All-Russian competition «Golden Names of Higher Education».

Such a person should be a real great scientist, teacher, master, person sparkling with talents. That's how we'll remember him.

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