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The Address of Editorial Office:
Russia, Moscow, 2nd Botkinskiy proezd, 3
Tel. 8 (495) 945–86–60
www: PDT-journal.com
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ORIGINAL ARTICLES

REVIEWS OF LITERATURE

ОРИГИНАЛЬНЫЕ СТАТЬИ

ОБЗОРЫ ЛИТЕРАТУРЫ

ORIGINAL ARTICLES

SPECTROSCOPIC STUDY OF METHYLENE BLUE IN VIVO: EFFECTS ON TISSUE OXYGENATION AND TUMOR METABOLISM

Pominova D.V.[1,2], Ryabova A.V.[1,2], Skobeltsin A.S[1], Markova I.V.[2], Romanishkin I.D.[1], Loschenov V.B.[1,2]
[1]Prokhorov General Physics Institute of Russian Academy of Sciences, Moscow, Russia
[2]National Research Nuclear University MEPhI (Moscow Engineering Physics Institute), Moscow, Russia

Abstract

Methylene blue (MB) is a promising photosensitizer (PS) for the treatment of pathological neoplasms, since it has both photodynamic activity (under laser irradiation) and redox and catalytic properties (in the absence of light). In the framework of this work, using spectroscopic methods, the effect of intravenous administration of MB on tissue oxygenation of hemoglobin in small animals in vivo in tumor and normal tissues was analyzed. The influence of MB on cell metabolism was analyzed. It has been shown that the use of MB promotes an increase in oxygen consumption by the tumor, and also leads to a shift in metabolism towards oxidative phosphorylation. It was shown that the use of MB contributes to an increase in oxygen consumption by the tumor, and also leads to a shift in metabolism towards oxidative phosphorylation.

Keywords: methylene blue, oxygenation, tumor metabolism.

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Contacts: Pominova D.V., e-mail: pominovadv@gmail.com

СПЕКТРОСКОПИЧЕСКОЕ ИССЛЕДОВАНИЕ МЕТИЛЕНОВОГО СИНЕГО IN VIVO: ВЛИЯНИЕ НА ОКСИГЕНАЦИЮ ТКАНЕЙ И ОПУХОЛЕВЫЙ МЕТАБОЛИЗМ

Д.В. Поминова[1,2], А.В. Рябова[1,2], А.С. Скобельцин[1], И.В. Маркова[2], И.Д. Романишкин[1], В.Б. Лощенов[1,2]
[1]Институт общей физики им. А. М. Прохорова Российской академии наук, Москва, Россия
[2]Национальный исследовательский ядерный университет «МИФИ», Москва, Россия

Резюме

Метиленовый синий (МС) является перспективным фотосенсибилизатором для терапии патологических новообразований, поскольку обладает как фотодинамической активностью (при лазерном облучении), так и окислительно-восстановительными и каталитическими свойствами (в отсутствии света). В рамках данной работы при помощи спектроскопических методов было проанализировано влияние внутривенного введения МС на тканевую оксигенацию гемоглобина на малых животных *in vivo* в опухоли и нормальных тканях. Проведен анализ влияния МС на клеточный метаболизм. Показано, что применение МС способствует увеличению потребления кислорода опухолью, а также приводит к сдвигу метаболизма в сторону окислительного фосфорилирования.

Ключевые слова: метиленовый синий, оксигенация, опухолевый метаболизм

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Контакты: Поминова Д.В.., e-mail: pominovadv@gmail.com

Introduction

Cancer is currently one of the major health problems in all developed and many developing countries of the world [1]. In this regard, a large number of publications are devoted to the study of the processes of tumor growth and their metastasis. To date, it is known that the tumor and its microenvironment are highly heterogeneous [2]. Cancer cells establish metabolic cross-talk with cellular and non-cellular components of the tumor microenvironment, which leads to the reorientation of immune cells to protect the tumor, provides cancer cells with the nutrients and promotes proliferation, invasion, metastasis, aggressiveness, resistance of tumors to treatment [3-6].

One of the driving forces of metabolic reprogramming is hypoxia and a cascade of biochemical reactions leading to local acidification. Growing tumors are characterized by insufficient blood perfusion, hypoxia, inflammation, enhanced fatty acid metabolism, nucleotide synthesis and glutaminolysis [7]. The hallmarks of tumor cell metabolism are a high level of glycolysis and a low level of oxidative phosphorylation, even when oxygen is present in the tissues in sufficient quantities. Most cancer cells produce lactic acid (lactate), a characteristic product of glycolysis [8]. Lactate has a critical function in signaling, through inducing the expression of vascular endothelial growth factor and the polarization of tumor-associated macrophages and induce expression of arginase 1 by macrophages, which has an important role in tumor growth [9]. Local acidity is a central regulator of cancer immunity that orchestrates both local and systemic immunosuppression [7]. Low oxygen supply to the tumor further enhances glycolysis, which in turn causes the expression of hypoxia-inducible factor 1α (HIF-1α) and mediates the effects of lactic acid.

An urgent task is to search for new approaches for the treatment of cancer, which are aimed at correcting the functional state of the tumor microenvironment [10-11]. One promising approach is photodynamic therapy (PDT) [12]. PDT uses a special drug–PS, which under the action of light generates reactive oxygen species that not only damage biological structures, but are also natural regulators of cell proliferation, metabolism, and apoptosis [13-14]. In recent years PDT has been increasingly used to treat tumors of various localizations. In Russia, a large number of scientific groups are engaged in the development of PDT methods [15-19]. PDT has a number of advantages over other methods: it is effective against all types of tumors; if necessary, the procedure can be repeated many times, since there are no cumulative toxic effects and acquired resistance; the procedure is carried out on an outpatient basis, provides a good cosmetic effect and can be used even for the elderly and debilitated people. The effectiveness and safety of PDT have been proven by numerous clinical studies and active practical use [20-22]. A problem for PDT is the effect on tumors that are in a state of hypoxia, for example, many tumors of the prostate and pancreas.

One of the interesting PS is MB, which, in addition to fluorescence in the red part of the spectrum and significant photodynamic activity, has redox and catalytic properties. In the 1930s, MB was actively researched [23-26] to counteract the effects of cyanide intoxication, however, after the advent of other antidotes [27], research on its mechanisms of action and effectiveness was abandoned for decades. According to a pioneering work [28], MB increases oxygen consumption by tissues with aerobic glycolysis and tumors, while the effect of MB is approximately proportional to the enzymatic capacity of tissues. There is no effect on oxygen consumption by those normal tissues that do not have aerobic glycolysis. The catalytic properties of MB in relation to tumors are due to its interaction with lactic acid, which is formed as a result of aerobic glycolysis.

When released into the blood, MB is readily reduced to its colorless leuco form, leucomethylene blue (LMB). Reducing agents can be NAD(P)H [29-30] or reduced glutathione [31], the concentration of which decreases as a result of interaction with MB [32], and MB acquires electrons in the process. LMB, in turn, can be reoxidized to MB by molecules with a higher redox potential (such as O_2 or most metal compounds), donating electrons in the process, and a new reduction cycle can be initiated [32]. There is evidence that MB interacts directly with the mitochondrial electronic circuit, donating electrons to complexes I and III and/or providing partial restoration of the Krebs cycle [33], whenever NADH is oxidized by MB or even resuscitation of the mitochondrial electronic circuit. A positive effect of MB on peripheral blood flow has also been reported [34]. In clinical practice, MB is used to treat methemoglobinemia, since MB is able to reduce the ferrous iron in methemoglobin (the oxidized form of hemoglobin that is unable to carry oxygen) to the ferric state corresponding to normal hemoglobin [35] and also as an antidote for carbon monoxide poisoning. The MB/LMB pair quickly diffuses into the cytoplasm and mitochondria of any cells, including neurons [36], and can have different effects depending on the concentration of the redox state of its immediate environment. MB and LMB have different absorption peaks: LMB predominantly absorb in the UV region (256 nm), while MB has two absorption peaks in the UV and visible range (294 and 665 nm, respectively) [37]. There is also a semi-reduced form (radical) with an absorption maximum at a wavelength of 420 nm. This makes it possible to study MBs by spectroscopic methods and directly observe the transition of MB into LMB.

It should be noted that early studies of MB were carried out *ex vivo* using micromanometric methods. More recent studies have mostly been conducted in cell cultures and have been indirect. Therefore, this work was devoted to the direct observation of the pharmacokinetics of MB *in vivo*, the study of the MB/LMB transition, and the assessment of the effect of MB on oxygenation and tumor metabolism using video fluorescence and spectroscopic methods. The present study was undertaken to characterize the metabolic responses to MB at various doses to determine the effects produced by the LMB/MB pair on cellular metabolism in a Lewis lung carcinoma (LLC) transplanted tumor mouse model. A spectroscopic study showed that with the accumulation of MB, there is a decrease in hemoglobin oxygenation in the tumor, which can be interpreted as an increase in oxygen consumption. Tissue cryosections were analyzed using fluorescence lifetime imaging microscopy (FLIM) in order to interpret intracellular metabolism in the areas of MB accumulation. It has been shown that there is a shift from glycolysis to oxidative phosphorylation after MB administration.

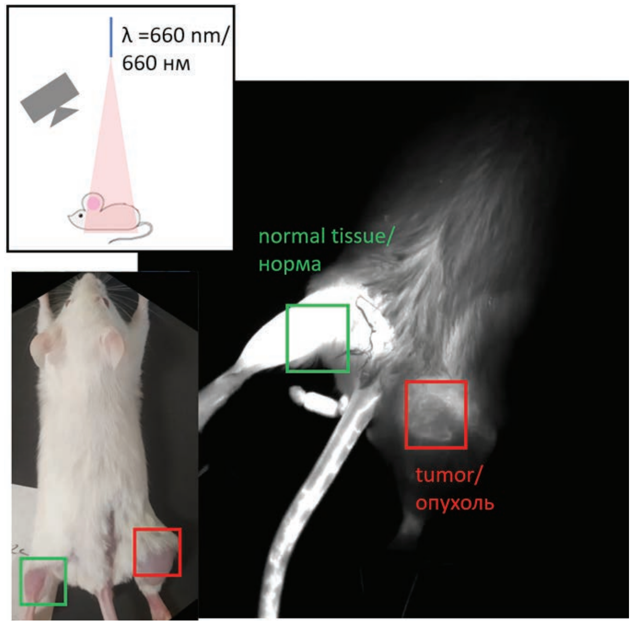

Рис. 1. Схематичное изображение расположения видеокамеры, источника лазерного излучения и мыши для флуоресцентной визуализации метиленового синего *in vivo*, фото животного с опухолью на правой лапе (красный квадрат) в обычном цвете и в флуоресцентном режиме.

Fig. 1. Schematic representation of the location of the video camera, laser source and mouse for fluorescence imaging of MB *in vivo*, photo of an animal with a tumor on the right paw (red square) in normal color and in fluorescent mode.

Materials and methods

Methylene blue

MB have been purchased at a pharmacy: "Methylene blue", an aqueous solution of 1%, the active substance methylthioninium chloride (OJSC "Samaramedprom").

Fluorescence imaging of MB in vivo

For the experiment, male BALB/c mice that were 25–30 g, 8–10 weeks old were used. The mice were kept at 21°C temperature in standard cages, the photoperiod was 12 hours of light and 12 hours of dark per day. The animals had access to standard laboratory feed and water *ad libitum*.

The LLC cell line of C57BL strain was used in experiments *in vivo* for tumor grafting. Inoculation of 50 μL of a 15% tumor cell suspension in Hanks' Balanced Salt Solution was performed intramuscularly on the right hind leg.

Experiments were performed on 14 after LLC cells injection. Tumor volume was determined by the measurement of two bisecting diameters in each tumor using calipers. The size of the tumor was determined by direct measurement of the tumor dimensions. The volume was calculated according to the equation: $V = (L \times W^2) \times 0.5$, where V = volume, L = length and W = width. All mice were divided into 2 groups depending on tumor size (small and large tumor, 50–75 and 100–150 mm^3 correspondingly). All measurements were triplicated.

MB was administered intravenously into the tail vein at a dose of 10 and 20 mg/kg with fluorescent control. Fluorescence was excited by laser radiation with a 660 nm wavelength.

Registration of fluorescent images was carried out using a black-and-white camera MQ013RG-ON (Ximea, Korea), with extended sensitivity in the near-infrared range, equipped with an interference filter that transmits in the range of 700-750 nm. The setup is shown in Fig. 1.

The fluorescent signal was recorded in a video file, which was further processed. After the injection of the dye, the mouse remained under laser irradiation for 5 minutes, during which the video file was recorded. The following method was used to assess pharmacokinetics from fluorescent images. For each frame of the recorded video file with a fluorescent signal, the average brightness in the specified area (tumor and normal tissue) was calculated. The brightness value in a pixel was normalized and took values from zero to one. Then, the time dependences of the average brightness of various zones of interest were plotted.

Study of methylene blue pharmacokinetics of using spectroscopic methods

Quantification of the MB accumulation in the tumor and in the normal tissue was carried out by spectroscopic methods using a fiber-optic spectrometer LESA-01-Biospec (Biospec, Russia). The device allows measurements of fluorescence spectra in the wavelength range of 350-1000 nm with a wavelength resolution of 3 nm. The exposure time for recording one spectrum can be varied in the range of 20 - 500 ms. To deliver and receive radiation, a fiber optic probe was used with a central illuminating fiber supplying exciting laser radiation to the tissue and six peripheral fibers collecting scattered and fluorescent radiation. A helium-neon laser with a wavelength of 632.8 nm was used to excite MB fluorescence. The laser radiation power at the output of the fiber was 5 mW. A filter was installed at the entrance to the spectrometer to attenuate the laser radiation, which made it possible to observe its component backscattered by the tissue in the same dynamic range as the fluorescent radiation.

The assessment of the concentration of the drug in the tissues using a fiber-optic spectrometer is performed integrally, from the entire depth to which the laser signal penetrates. To quantitatively determine the MB concentration in organs and tissues, the calibration was performed using optical phantoms with an MB photosensitizer, which simulated the scattering and absorbing properties of biological tissues. Used MB concentrations of 0, 0.01, 0.05, 0.1, 0.5, 1, 2.5, 5 mg/kg were mixed with a scattering medium (1% fat emulsion Intralipid (Fresenius Kabi LLC, USA)) and put into tubes. The fluorescence spectra of optical phantoms with MB were recorded under excitation by a laser source with a wavelength of 632.8 nm. Based on the obtained spectra, the fluorescence index was determined for each optical phantom, equal to the ratio of the area under the MB fluorescence peak to the area under the scattered laser radiation peak. Using the calibration curve, a one-to-one correspondence is established between the fluorescence index of each of the phantoms and the concentration of MB in it. The calibration curve is then used to determine the concentration of MB *in vivo* from their measured fluorescence indices. Fluorescence index and MB concentration in optical phantoms were determined under the same external conditions. All measurements were carried out in a dark room without external light sources. Measurements were made at time points prior to administration, 10 minutes and 1 hour. For each time point, measurements were repeated three times for 3 animals.

Determination of hemoglobin oxygenation level in the tissue microvasculature by analyzing the diffuse reflectance spectra

The hemoglobin oxygenation measurement is based on the registration of diffuse reflectance spectra in the 500-600 nm wavelength spectral range, which makes it possible to quantify the concentration of hemoglobin in oxygenated and deoxygenated form. Fig. 2 shows the sketch of the experimental setup for diffuse reflectance spectra registration *in vivo* (Fig. 2a) and characteristic absorption spectra of oxygenated and deoxygenated hemoglobin forms (Fig. 2b).

A halogen lamp with a fiber optic output was used as a broadband radiation source. To receive radiation, a fiber with a diameter of 400 µm was used. The light supplied to the biological object passed through the tissue, experiencing scattering and absorption, and entered the receiving fiber. The receiving and transmitting fibers were in light contact with the test tissue in order to avoid affecting its optical properties when pressed. From the receiving fiber, light entered the LESA-01-BIOSPEC

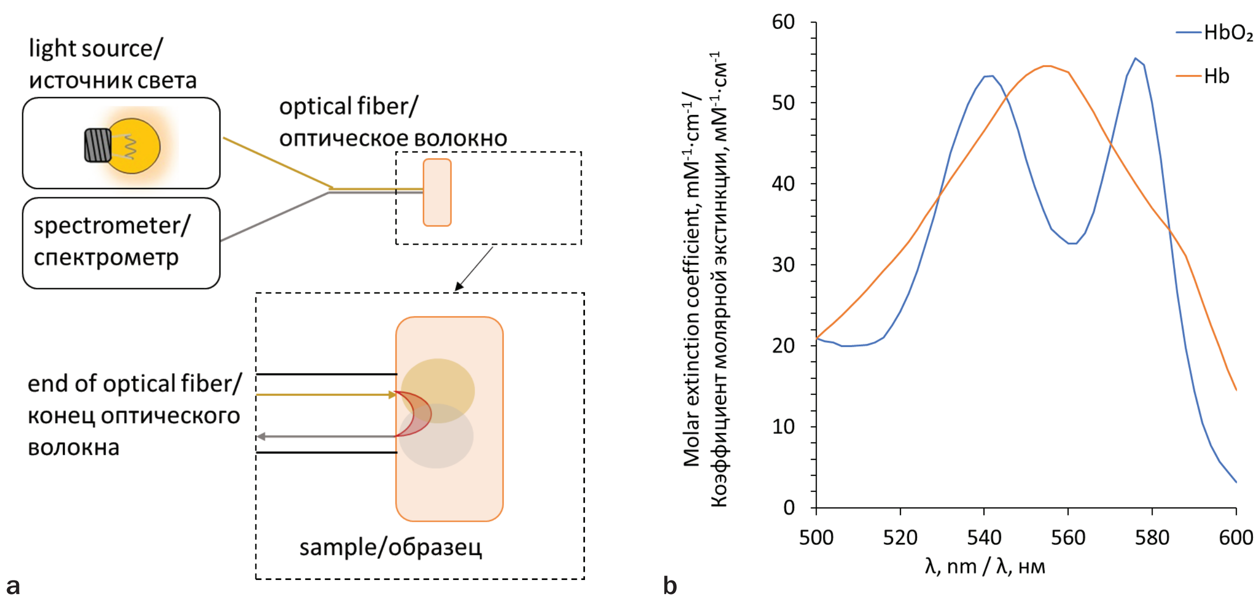

Рис. 2. Схема экспериментальной установки для измерения спектров обратного диффузного рассеяния *in vivo* (a) и характеристические спектры поглощения оксигенированной и деоксигенированной форм гемоглобина (b).
Fig. 2. Scheme of the experimental setup for measuring diffuse reflectance spectra *in vivo* (a) and characteristic absorption spectra of oxygenated and deoxygenated hemoglobin forms (b).

laser spectrum analyzer (Biospec, Russia), which was controlled via a USB interface by a personal computer using special software Uno (Biospec, Russia), which was used to register and process the spectral dependencies. To eliminate the influence of the spectral sensitivity of the detector, the transmission spectrum of the fibers, and the spectral radiative characteristic of the light source on the detected signal, the measurements were carried out relative to a standard sample ($BaSO_4$) with a reflection coefficient close to unity in the spectral range of interest. The measurement technique is described in more detail in [38].

Cryosections preparation and analysis

After spectroscopic study mice were euthanized. Tumors along with subcutaneous tissue, skin and muscle were excised en bloc and frozen. Using a freezing microtome Microm HM 560 Cryostat (Thermo Scientific, Waltham, Massachusetts, USA) cryosections were prepared. Thickness was estimated to be 50 μm for the FLIM procedure and 100 μm for absorption spectra measurements. The sections were placed in saline under a coverslip and examined immediately using a laser scanning microscope in order to analyze the metabolic changes after MB accumulation. To study the absorption spectra, the sections were placed on quartz glasses. Registration of absorption spectra in the range of 200-1000 nm was carried out using a Hitachi U3400 spectrophotometer (Hitachi, Japan).

Assessment of intracellular metabolism by endogenous NADH photoluminescence lifetime using FLIM

To investigate the metabolic changes in the tissue, an approach based on calculating NADH fluorescence lifetime metabolic index was used [39]. Tissue sections were examined using an LSM-710-NLO laser scanning microscope (Carl Zeiss AG, Germany) with Plan-Apochromat 63x/1.4 Oil objective. NADH fluorescence was excited by 740 nm two-photon laser excitation using Chameleon Ultra II femtosecond laser (Coherent, USA). Time-resolved images were obtained using an attached FLIM module (Becker & Hickl GmbH, Berlin, Germany) consisting of a time-correlated single photon counting system SPC-150, a GaAsP HPM-100-07 hybrid photodetector, and SPCM software the fluorescence lifetime was measured. NADH fluorescence was isolated using an FB450-40 bandpass optical filter (Thorlabs, USA).

Time-resolved fluorescence images were processed using SPCImage 8.5 software (Becker & Hickl GmbH, Germany). To interpret the time-resolved fluorescence, NADH a_1/a_2 metabolic index was calculated for each pixel of the image, where a_1 and a_2 are amplitudes of the short (τ_1 = 0.4 ns) and long (τ_2 = 2.5 ns) lifetime components of free and bound NADH, respectively [40]. High values of the metabolic index signify the shift of cellular metabolism towards glycolysis, while low values—towards oxidative phosphorylation. In addition to calculating the metabolic index, a phasor diagram approach was applied [41].

Results and discussions

Fluorescence imaging and spectroscopic studies of methylene blue in vivo

Using the video fluorescence imaging, it was shown that after intravenous administration, MB accumulates very quickly (in about 5 seconds) both in the tumor and in normal tissue, Fig. 3.

Then, the intensity of MB fluorescence in normal tissue decreases slightly and remains constant throughout the measurement (5 minutes). In a tumor, on the contrary, a rapid decrease in the MB fluorescence intensity is observed; already after 20 seconds, the luminescence intensity decreases by 4 times relative to the initial value and remains at this level. The obtained time dependences

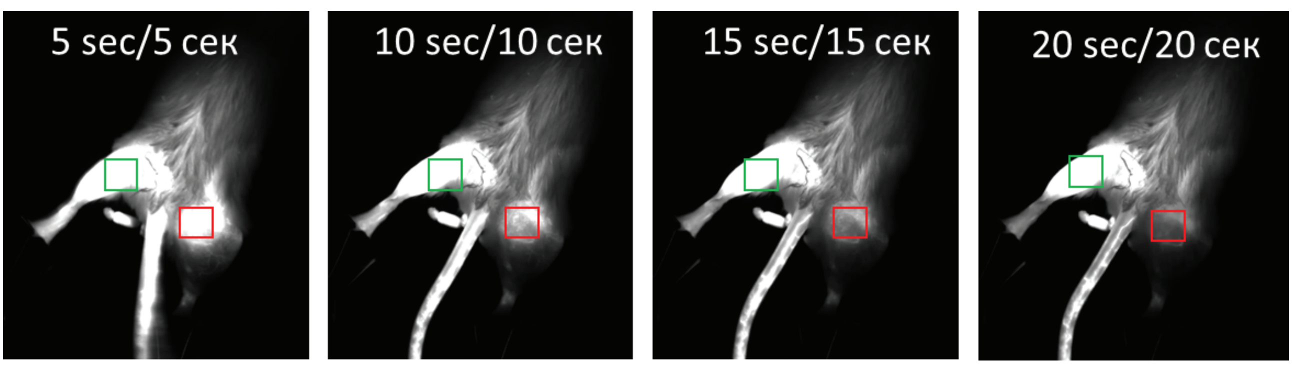

Рис. 3. Флуоресцентная визуализация метиленового синего *in vivo* с использованием возбуждения 660 нм: изображения, полученные через 5, 10, 15 и 20 с после внутривенного введения метиленового синего в дозе 20 мг/кг. Область, выделенная зеленым, соответствует нормальной ткани, область, выделенная красным, – опухоли.
Fig. 3. Fluorescence imaging of MB *in vivo* using 660 nm excitation: images obtained 5, 10, 15 and 20 seconds after intravenous injection MB in 20 mg/kg dose. The area highlighted in green corresponds to normal tissue, the area highlighted in red corresponds to tumor.

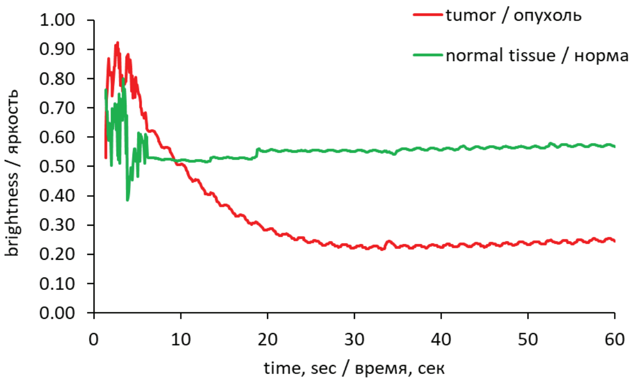

Рис. 4. Зависимости средней яркости для выбранных областей нормальных тканей и опухоли на флуоресцентном изображении от времени.
Fig. 4. Time dependences of the fluorescent image average brightness of selected normal tissue and tumor areas.

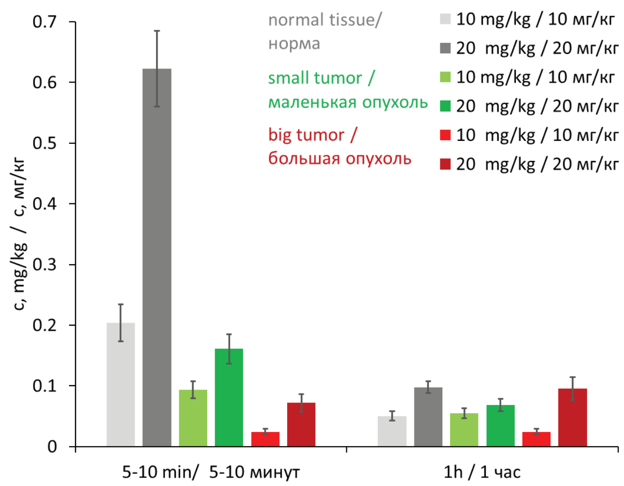

Рис. 5. Концентрация метиленового синего в нормальных тканях и опухоли, определенная спектроскопическими методами через 5 мин и 1 ч после внутривенного введения в дозе 10 и 20 мг/кг.
Fig. 5. The concentration of MB in normal tissues and tumors, determined by spectroscopic methods 5 minutes and 1 hour after intravenous administration at a dose of 10 and 20 mg/kg.

of the average brightness of normal tissue and tumor areas are presented on Fig. 4.

A large spread in fluorescence intensity values in the interval of 2–6 seconds is associated with mouse movement at the moment of MB injection and immediately after. We assume that the observed decrease in the intensity of MB fluorescence is due to the rapid transition to the reduced LMB due to interaction with the components of the tumor microenvironment.

Quantitative assessment of MB accumulation in normal tissue and tumor was carried out using spectroscopic methods by the intensity of fluorescence in the red spectral range, recorded *in vivo*. The dependence of MB concentration on the accumulation time is shown in Fig. 5.

The maximum accumulation in the normal tissue and small tumor was observed 5–10 minutes after injection for both tested concentrations. As expected, the MB cumulative concentration was higher for the higher MB dose. An hour later, the concentration decreased significantly, both in the norm and in a small tumor. For a large tumor, the opposite trend was observed – with an increase in the accumulation time, the MB concentration gradually increased.

To confirm that the decrease in the luminescence intensity of MB is due to the transition to the reduced form of LMB, we studied the absorption of cryosections of normal and tumor tissues *ex vivo*. The absorption spectra recorded using a spectrophotometer are shown in Fig. 6.

The study of the absorption spectra of cryosections *ex vivo* showed the presence of the transition of MB to LMB in the tumor. In the absorption spectrum of normal tissue, an absorption peak is observed in the red region, corresponding to the absorption of the "blue" form. The absorption spectrum of the tumor lacks a peak in the red region, but there is intense absorption in the UV range, presumably corresponding to the absorption of LMB. The absorption peak at a wavelength of 420 nm

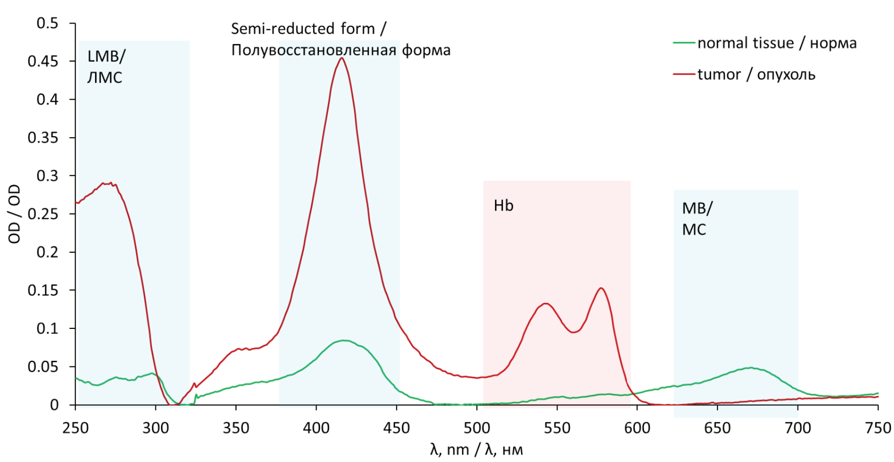

Рис. 6. Спектры поглощения криосрезов нормальных и опухолевых тканей после введения метиленового синего, доза 20 мг/кг (МС – метиленовый синий, ЛМС – лейкометиленовый синий).
Fig. 6. Absorption spectra of normal and tumor tissues cryosections obtained after MB injection, dose 20 mg/kg.

corresponds to the absorption of the semi-reduced form; in the range of 500–600 nm, a characteristic hemoglobin absorption peak is observed for the tumor. Thus, analysis of the absorption spectra makes it possible to study the transition of the main form of MB into its reduced form under the influence of external factors.

Determination of hemoglobin oxygenation level in the tissue microvasculature

Along with assessment of MB accumulation in normal tissue and tumor, the oxygenation level *in vivo* was measured by the hemoglobin absorption. The dependence of tumor oxygenation in relation to normal tissue on the MB accumulation time is shown in Fig. 7.

Prior to the administration of MB, the degree of tumor oxygenation is about 85 and 70% relative to normal tissues for small and large tumors, respectively. In 5–10 minutes after the administration of MB for small tumors, a decrease in the level by 10% is observed, 1 hour after the administration, oxygenation is restored to its original level or exceeds it, depending on the concentration of the drug. 5 hours after the administration of the drug for small tumors, the degree of oxygenation continues to increase and approaches the level of oxygenation of normal tissues (90%). This dependence correlates with the pharmacokinetics of MB in small tumors: after 5 minutes, the maximum accumulation of MB (in the oxidized «blue» form) is observed, after an hour the concentration of the drug decreases.

The dependence of oxygenation of large tissues on time after the introduction of MB has a different character. Initially, oxygenation of large tumors is lower and is about 70% of the norm. 5-10 minutes after the administration of MB, an increase in oxygenation up to 90% is observed, and then oxygenation begins to decrease and is about 65 and 40% 1 and 5 hours after administration, respectively.

According to literature data, MB increases oxygen consumption by tissues with aerobic glycolysis [28]. In this case, oxygen consumption is understood as the amount of oxygen absorbed and used by the body per minute, that is, this is the rate of oxygen use. From the point of view of oxygen consumption, the obtained dependences of the oxygenation on time can be interpreted as follows. For small tumors, MB accumulates rapidly in the tumor and increases oxygen uptake. At the same time, oxygenation of hemoglobin in the microvasculature in the tumor area is reduced. After a while, the concentration of MB decreases and oxygenation begins to increase. At the same time, a temporary increase in oxygen consumption leads to the increase of tumor oxygenation after exposure, which exceeds the initial one. For large tumors, MB accumulation is slower and the concentration accumulated in the tumor is significantly lower than in small tumors (Fig. 5), since the central part of the tumor is poorly supplied with blood. A decrease in oxygenation is observed after a longer time after the administration of the drug (Fig. 7) since more time is required for MB to accumulate in tumor tissues and have an effect. The increase in the oxygenation level after 5–10 minutes can be explained by the positive effect of MB on peripheral blood flow.

Assessment of intracellular metabolism by endogenous NADH photoluminescence lifetime

Time-resolved fluorescence imaging was used to investigate the effect of MB administration on the metabolic type of tumor tissues. Phasor diagrams in the NADH spectral range from tumor slices after MB intravenous injection in 20 mg/kg dose are shown in Fig. 8.

The phasor diagrams of tumors treated with MB show a shift towards shorter lifetimes relative to the control tumor. The NADH a_1/a_2 metabolic index calculated from

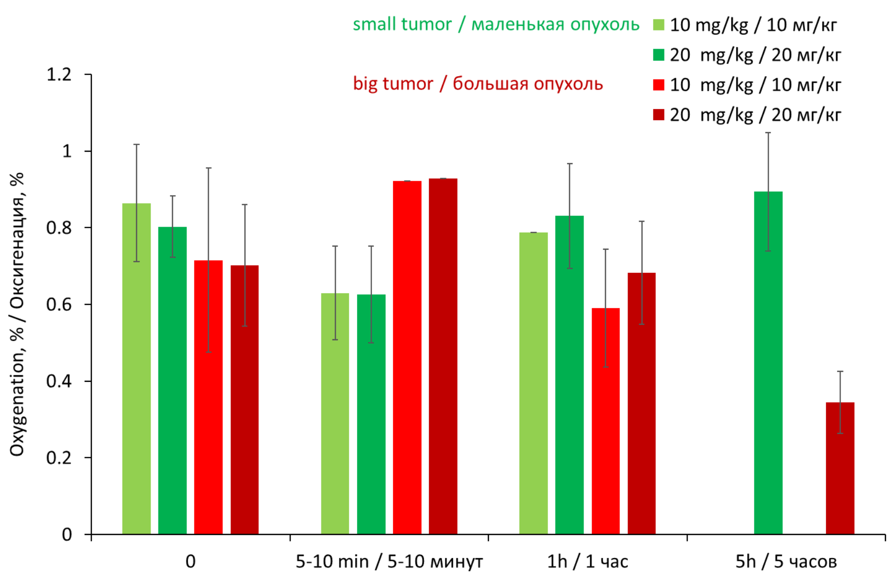

Рис. 7. Степень оксигенации опухоли по отношению к нормальной ткани, определенная по поглощению гемоглобина до, через 5 мин и через 1 ч после внутривенного введения метиленового синего в дозе 10 и 20 мг/кг.

Fig. 7. The oxygenation level of tumor in relation to normal tissue, determined by the hemoglobin absorption before, and 5 minutes and 1 hour after MB intravenous administration at a dose of 10 and 20 mg/kg.

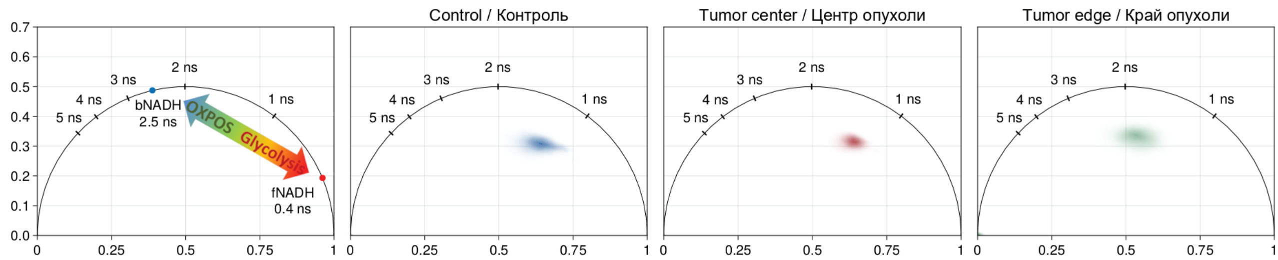

Рис. 8. Фазорные диаграммы разрешенных во времени флуоресцентных изображений NADH в срезах опухоли после внутривенного введения метиленового синего в дозе 20 мг/кг: контроль – опухоль без метиленового синего, центр опухоли – измерение в центре опухоли, край опухоли – измерение на краю опухоли.
Fig. 8. Phasor diagrams for time-resolved fluorescence images of NADH in tumor cryosections after MB intravenous injection in 20 mg/kg dose: control – tumor without MB, tumor center – measurement in the center of the tumor, tumor edge – measurement at the edge of the tumor.

slice images amounted to 8.01±1.84, 7.12±0.87 and 6.65±1.56 for the control tumor without MB, the center and periphery of the tumor with MB, respectively. Such a shift in the metabolic index indicates a change in the type of metabolism from glycolysis to oxidative phosphorylation.

The difference in the results for the center and periphery of the tumor is due to the fact that the blood vessels that deliver oxygen and MB to the tumor, identified in general toward the epithelial surface but not intertwined deep into the tumor bulk [42]. For tumors, there is usually a decrease in the gradient of oxygen and nutrients from the periphery to the center. Thus, for the periphery of the tumor, there is a higher accumulation of MB due to a better blood supply, as well as a better supply of oxygen.

Conclusion

Using fluorescent imaging and spectroscopic methods, the accumulation of MB in tumors *in vivo* was studied, its effect on the hemoglobin oxygenation level in the tissue microvasculature, as well as tumor metabolism, were analyzed.

After intravenous administration, a rapid decrease in the MB fluorescence intensity was observed in the tumor. After 20 seconds, the luminescence intensity decreases by 4 times relative to the initial value and remains at this level. We assume that the observed decrease in the intensity of MB fluorescence is due to the rapid transition to the reduced LMB due to interaction with the components of the tumor microenvironment. This assumption is confirmed by the absorption spectra of cryosections *ex vivo* showing the presence of the transition of MB to LMB in the tumor. Intense absorption in the UV range, presumably corresponding to the absorption of LMB, was observed in the absorption spectrum of the tumor.

For small tumors, MB accumulates rapidly in the tumor and increases oxygen uptake, so the oxygenation of hemoglobin in the microvasculature in the tumor area decreases. When the concentration of methylene blue decreases and oxygenation begins to increase. At the same time, a temporary increase in oxygen consumption leads to the increase of tumor oxygenation after exposure, which exceeds the initial one. For large tumors, MB accumulation is slower, so the decrease in oxygenation is observed after a longer time after the administration of the drug.

The phasor diagrams of tumors treated with MB show a shift towards shorter lifetimes relative to the control tumor. The NADH a_1/a_2 metabolic index calculated from slice images amounted to 8.01±1.84, 7.12±0.87 and 6.65±1.56 for the control without MB, the center and periphery of the tumor with MB, respectively. Such a shift in the metabolic index indicates a change in the type of metabolism from glycolysis to oxidative phosphorylation.

Thus, the use of MB contributes to an increase in oxygen consumption by the tumor, and also leads to a shift in metabolism towards oxidative phosphorylation. We assume that this will positively influence the tumor microenvironment towards tumor regression. Increasing tissue oxygenation with the help of MB will significantly increase the effectiveness of PDT. The results obtained have great potential for practical implementation, as they will significantly increase the effectiveness of modern methods of cancer therapy, especially in relation to tumors in a state of hypoxia that are difficult to treat.

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REFERENCES

1. Bray F., Ferlay J., Soerjomataram I., Siegel R.L., Torre L.A., Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, *Global cancer statistics*, 2018, 6, pp. 394-424.
2. Biswas S.K., Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm, *Nature*

ЛИТЕРАТУРА

1. Bray F., Ferlay J., Soerjomataram I., Siegel R.L., Torre L.A., Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries // Global cancer statistics 2018. – № 6. – P. 394-424.
2. Biswas S.K., Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm // Nature

Immunology, 2010, Vol. 11(10), pp. 889-896.
3. Pranzini E., Pardella E., Paoli P., Fendt S.-M., Taddei M.L. Metabolic Reprogramming in Anticancer Drug Resistance: A Focus on Amino Acids, *Trends in Cancer*, 2021, Vol. 7(8), pp. 682-699.
4. Persi E., Duran-Frigola M., Damaghi M., Roush W.R., Aloy P., Cleveland J.L., Gillies R.J., Ruppin E. Systems analysis of intracellular pH vulnerabilities for cancer therapy, *Nature Communications*, 2018, Vol. 9(1).
5. Chen D., Xie J., Fiskesund R., Dong W., Liang X., Lv J., Jin X., Liu J., Mo S., Zhang T., Cheng F., Zhou Y., Zhang H., Tang K., Ma J., Liu Y., Huang B. Chloroquine modulates antitumor immune response by resetting tumor-associated macrophages toward M1 phenotype, *Nature Communications*, 2018, Vol. 9(1).
6. Wu A.A., Drake V., Huang H.-S., Chiu S., Zheng L. Reprogramming the tumor microenvironment: tumor-induced immunosuppressive factors paralyze T cells, *OncoImmunology*, 2015, Vol. 4(7), pp. e1016700.
7. Huber V., Camisaschi C., Berzi A., Ferro S., Lugini L., Triulzi T., Tuccitto A., Tagliabue E., Castelli C., Rivoltini L. Cancer acidity: An ultimate frontier of tumor immune escape and a novel target of immunomodulation, *Seminars in Cancer Biology*, 2017, Vol. 43, pp. 74-89.
8. Vander Heiden M.G., Cantley L.C., Thompson C.B. Understanding the Warburg Effect: The Metabolic Requirements of Cell Proliferation, *Science*, 2009, Vol. 324(5930), pp. 1029-1033.
9. Colegio O.R., Chu N.-Q., Szabo A.L., Chu T., Rhebergen A.M., Jairam V., Cyrus N., Brokowski C.E., Eisenbarth S.C., Phillips G.M., Cline G.W., Phillips A.J., Medzhitov R. Functional polarization of tumour-associated macrophages by tumour-derived lactic acid, *Nature*, 2014, Vol. 513(7519), pp. 559-563.
10. 2018 Nobel Prize in Physiology or Medicine James pp. Allison and Tasuku Honjo "For their discovery of cancer therapy by inhibition of negative immune regulation"
11. Blaylock R. Cancer microenvironment, inflammation and cancer stem cells: A hypothesis for a paradigm change and new targets in cancer control, *Surgical Neurology International*, 2015, Vol. 6(1), pp. 92.
12. Correia J.H., Rodrigues J.A., Pimenta S., Dong T., Yang Z. Photodynamic Therapy Review: Principles, Photosensitizers, Applications, and Future Directions, *Pharmaceutics*, 2021, Vol. 13(9), pp. 1332.
13. Fleury C., Mignotte B., Vayssière J.-L. Mitochondrial reactive oxygen species in cell death signaling, *Biochimie*, 2002, Vol. 84(2-3), pp. 131-141.
14. Villalpando-Rodriguez G.E., Gibson S.B. Reactive Oxygen Species (ROS) Regulates Different Types of Cell Death by Acting as a Rheostat, *Oxidative Medicine and Cellular Longevity*, 2021, Vol. 2021, pp. 1-17.
15. Kuznetsov V.V. The use of photod ynamic therapy in the domestic oncology (Review of the literature), *Research'n Practical Medicine Journal*, 2016, Vol. 2(4), pp. 98-105.
16. Reshetov I.V., Korenev S.V., Romanko Yu.S. Modern aspects of photodynamic therapy of basal cell skin cancer, *Biomedical Photonics*, 2022, Vol. 11(3), pp. 35-39.
17. Stranadko E.F. Main stages of development of photodynamic therapy in Russia, *Biomedical Photonics*, 2015, Vol. 4(1), pp. 3-10.
18. Panaseykin Y.A., Kapinus V.N., Filonenko E.V., Polkin V.V., Sevrukov F.E., Isaev P.A., Ivanov S.A., Kaprin A.D. Photodynamic therapy treatment of oral cavity cancer in patients with comorbidities, *Biomedical Photonics*, 2023, Vol. 11(4), pp. 19-24.
19. Klimenko V.V., Knyazev N.A., Moiseenko F.V., Rusanov A.A., Bogdanov A.A., Dubina M.V. Pulse mode of laser photodynamic treatment induced cell apoptosis, *Photodiagnosis and Photodynamic Therapy*, 2016, Vol. 13, pp. 101-107.
20. Mansoori B., Mohammadi A., Amin Doustvandi M., Mohammadnejad F., Kamari F., Gjerstorff M.F., Baradaran B., Hamblin M.R. Photodynamic therapy for cancer: Role of natural products, *Photodiagnosis and Photodynamic Therapy*, 2019, Vol. 26, pp. 395-404.
21. Spring B.Q., Rizvi I., Xu N., Hasan T. The role of photodynamic therapy in overcoming cancer drug resistance, *Photochemical & Photobiological Sciences*, 2015, Vol. 14(8), pp. 1476-1491.
22. Brown S.B., Brown E.A., Walker I. The present and future role of photodynamic therapy in cancer treatment, *The Lancet Oncology*, 2004, Vol. 5(8), pp. 497-508.
23. Brooks M.M. The Mechanism of Methylene Blue Action on Blood, *Science*, 1934, Vol. 80(2062), pp. 15-16.

Immunology. – 2010. – Vol. 11. – Macrophage plasticity and interaction with lymphocyte subsets. – № 10. – P. 889-896.
3. Pranzini E., Pardella E., Paoli P., Fendt S.-M., Taddei M.L. Metabolic Reprogramming in Anticancer Drug Resistance: A Focus on Amino Acids // Trends in Cancer. – 2021. – Vol. 7. – Metabolic Reprogramming in Anticancer Drug Resistance. – № 8. – P. 682-699.
4. Persi E., Duran-Frigola M., Damaghi M., Roush W.R., Aloy P., Cleveland J.L., Gillies R.J., Ruppin E. Systems analysis of intracellular pH vulnerabilities for cancer therapy // Nature Communications. – 2018. – Vol. 9. – № 1.
5. Chen D., Xie J., Fiskesund R., Dong W., Liang X., Lv J., Jin X., Liu J., Mo S., Zhang T., Cheng F., Zhou Y., Zhang H., Tang K., Ma J., Liu Y., Huang B. Chloroquine modulates antitumor immune response by resetting tumor-associated macrophages toward M1 phenotype// Nature Communications. – 2018. – Vol. 9. – № 1.
6. Wu A.A., Drake V., Huang H.-S., Chiu S., Zheng L. Reprogramming the tumor microenvironment: tumor-induced immunosuppressive factors paralyze T cells // OncoImmunology. – 2015. – Vol. 4. – Reprogramming the tumor microenvironment. – № 7. – P. e1016700.
7. Huber V., Camisaschi C., Berzi A., Ferro S., Lugini L., Triulzi T., Tuccitto A., Tagliabue E., Castelli C., Rivoltini L. Cancer acidity: An ultimate frontier of tumor immune escape and a novel target of immunomodulation // Seminars in Cancer Biology. – 2017. – Vol. 43. – Cancer acidity. – P. 74-89.
8. Vander Heiden M.G., Cantley L.C., Thompson C.B. Understanding the Warburg Effect: The Metabolic Requirements of Cell Proliferation // Science. – 2009. – Vol. 324. – Understanding the Warburg Effect. – № 5930. – P. 1029-1033.
9. Colegio O.R., Chu N.-Q., Szabo A.L., Chu T., Rhebergen A.M., Jairam V., Cyrus N., Brokowski C.E., Eisenbarth S.C., Phillips G.M., Cline G.W., Phillips A.J., Medzhitov R. Functional polarization of tumour-associated macrophages by tumour-derived lactic acid // Nature. – 2014. – Vol. 513. – № 7519. – P. 559-563.
10. 2018 Nobel Prize in Physiology or Medicine James pp. Allison and Tasuku Honjo "For their discovery of cancer therapy by inhibition of negative immune regulation"
11. Blaylock R. Cancer microenvironment, inflammation and cancer stem cells: A hypothesis for a paradigm change and new targets in cancer control // Surgical Neurology International. – 2015. – Vol. 6. – Cancer microenvironment, inflammation and cancer stem cells. – № 1. – P. 92.
12. Correia J.H., Rodrigues J.A., Pimenta S., Dong T., Yang Z. Photodynamic Therapy Review: Principles, Photosensitizers, Applications, and Future Directions // Pharmaceutics. – 2021. – Vol. 13. – Photodynamic Therapy Review. – № 9. – P. 1332.
13. Fleury C., Mignotte B., Vayssière J.-L. Mitochondrial reactive oxygen species in cell death signaling // Biochimie. – 2002. – Vol. 84. – № 2-3. – P. 131-141.
14. Villalpando-Rodriguez G.E., Gibson S.B. Reactive Oxygen Species (ROS) Regulates Different Types of Cell Death by Acting as a Rheostat // Oxidative Medicine and Cellular Longevity. – 2021. – Vol. 2021. – P. 1-17.
15. Kuznetsov V.V. The use of photod ynamic therapy in the domestic oncology (Review of the literature) // Research'n Practical Medicine Journal. – 2016. – Vol. 2. – № 4. – P. 98-105.
16. Reshetov I.V., Korenev S.V., Romanko Yu.S. Modern aspects of photodynamic therapy of basal cell skin cancer // Biomedical Photonics. – 2022. – Vol. 11. – № 3. – P. 35-39.
17. Stranadko E.F. Main stages of development of photodynamic therapy in Russia // Biomedical Photonics. – 2015. – Vol. 4. – № 1. – P. 3-10.
18. Panaseykin Y.A., Kapinus V.N., Filonenko E.V., Polkin V.V., Sevrukov F.E., Isaev P.A., Ivanov S.A., Kaprin A.D. Photodynamic therapy treatment of oral cavity cancer in patients with comorbidities // Biomedical Photonics. – 2023. – Vol. 11. – № 4. – P. 19-24.
19. Klimenko V.V., Knyazev N.A., Moiseenko F.V., Rusanov A.A., Bogdanov A.A., Dubina M.V. Pulse mode of laser photodynamic treatment induced cell apoptosis // Photodiagnosis and Photodynamic Therapy. – 2016. – Vol. 13. – P. 101-107.
20. Mansoori B., Mohammadi A., Amin Doustvandi M., Mohammadnejad F., Kamari F., Gjerstorff M.F., Baradaran B., Hamblin M.R. Photodynamic therapy for cancer: Role of natural products // Photodiagnosis and Photodynamic Therapy. – 2019. – Vol. 26. – Photodynamic therapy for cancer. – P. 395-404.
21. Spring B.Q., Rizvi I., Xu N., Hasan T. The role of photodynamic therapy in overcoming cancer drug resistance // Photochemical & Photobiological Sciences. – 2015. – Vol. 14. – № 8. – P. 1476-1491.
22. Brown S.B., Brown E.A., Walker I. The present and future role of photodynamic therapy in cancer treatment // The Lancet Oncology. – 2004. – Vol. 5. – № 8. – P. 497-508.
23. Brooks M.M. The Mechanism of Methylene Blue Action on Blood // Science. – 1934. – Vol. 80. – № 2062. – P. 15-16.

24. Brooks M.M. Methylene blue as antidote for cyanide and carbon monoxide poisoning, *JAMA: The Journal of the American Medical Association*, 1933, Vol. 100(1), pp. 59.
25. Wendel W.B. The Mechanism of the Antidotal Action of Methylene Blue in Cyanide Poisoning, *Science*, 1934, Vol. 80(2078), pp. 381-382.
26. Wendel W.B. The control of methemoglobinemia with methylene blue, *Journal of Clinical Investigation*, 1939, Vol. 18(2), pp. 179-185.
27. Chen K.K. Nitrite and thiosulfate therapy in cyanide poisoning, *Journal of the American Medical Association*, 1952, Vol. 149(2), pp. 113.
28. Barron E.S.G. The catalytic effect of methylene blue on the oxygen consumption of tumors and normal tissues, *Journal of Experimental Medicine*, 1930, Vol. 52(3), pp. 447-456.
29. Sevcik P., Dunford H.B. Kinetics of the oxidation of NADH by methylene blue in a closed system, *The Journal of Physical Chemistry*, 1991, Vol. 95(6), pp. 2411-2415.
30. Engbersen J.F.J., Koudijs A., Van Der Plas H.C. Reaction of NADH models with methylene blue, *Recueil des Travaux Chimiques des Pays-Bas*, 2010, Vol. 104(5), pp. 131-138.
31. Schirmer R.H., Adler H., Pickhardt M., Mandelkow E. "Lest we forget you — methylene blue …", *Neurobiology of Aging*, 2011, Vol. 32(12), pp. 2325.e7-2325.e16.
32. Buchholz K., Schirmer R.H., Eubel J.K., Akoachere M.B., Dandekar T., Becker K., Gromer S. Interactions of Methylene Blue with Human Disulfide Reductases and Their Orthologues from Plasmodium falciparum, *Antimicrobial Agents and Chemotherapy*, 2008, Vol. 52(1), pp. 183-191.
33. Komlódi T., Tretter L. Methylene blue stimulates substrate-level phosphorylation catalysed by succinyl–CoA ligase in the citric acid cycle, *Neuropharmacology*, 2017, Vol. 123, pp. 287-298.
34. Zhang H., Rogiers P., Preiser J.-C., Spapen H., Manikis P., Metz G., Vincent J.-L. Effects of methylene blue on oxygen availability and regional blood flow during endotoxic shock:, *Critical Care Medicine*, 1995, Vol. 23(10), pp. 1711-1721.
35. Tepaev R.F., Vishnevskiy V.A., Kuzin S.A., Sergey I.V., Gordeeva O.B., Pytal A.V., Murashkin N.N. Benzocaine-Induced Methemoglobinemia. A Clinical Case, *Pediatric pharmacology*, 2018, Vol. 15(5), pp. 396-401.
36. Peter C., Hongwan D., Küpfer A., Lauterburg B.H. Pharmacokinetics and organ distribution of intravenous and oral methylene blue, *European Journal of Clinical Pharmacology*, 2000, Vol. 56(3), pp. 247-250.
37. Lee S.-K., Mills A. Novel photochemistry of leuco-Methylene Blue, *Chemical Communications*, 2003, 18, pp. 2366.
38. Stratonnikov A.A., Loschenov V.B. Evaluation of blood oxygen saturation in vivo from diffuse reflectance spectra, *Journal of Biomedical Optics*, 2001, Vol. 6(4), pp. 457.
39. Sharick J.T., Favreau P.F., Gillette A.A., Sdao S.M., Merrins M.J., Skala M.C. Protein-bound NAD(P)H Lifetime is Sensitive to Multiple Fates of Glucose Carbon, *Scientific Reports*, 2018, Vol. 8(1), pp. 5456.
40. Kalinina S., Freymueller C., Naskar N., Von Einem B., Reess K., Sroka R., Rueck A. Bioenergetic Alterations of Metabolic Redox Coenzymes as NADH, FAD and FMN by Means of Fluorescence Lifetime Imaging Techniques, *International Journal of Molecular Sciences*, 2021, Vol. 22(11), pp. 5952.
41. Ranjit S., Malacrida L., Jameson D.M., Gratton E. Fit-free analysis of fluorescence lifetime imaging data using the phasor approach, *Nature Protocols*, 2018, Vol. 13(9), pp. 1979-2004.
42. Worth K.R., Papandreou I., Hammond E.M. How the histological structure of some lung cancers shaped almost 70 years of radiobiology, *British Journal of Cancer*, 2023, Vol. 128(3), pp. 407-412.

24. Brooks M.M. Methylene blue as antidote for cyanide and carbon monoxide poisoning // JAMA: The Journal of the American Medical Association. – 1933. – Vol. 100. – № 1. – P. 59.
25. Wendel W.B. The Mechanism of the Antidotal Action of Methylene Blue in Cyanide Poisoning // Science. – 1934. – Vol. 80. – № 2078. – P. 381-382.
26. Wendel W.B. The control of methemoglobinemia with methylene blue // Journal of Clinical Investigation. – 1939. – Vol. 18. – № 2. – P. 179-185.
27. Chen K.K. Nitrite and thiosulfate therapy in cyanide poisoning // Journal of the American Medical Association. – 1952. – Vol. 149. – № 2. – P. 113.
28. Barron E.S.G. The catalytic effect of methylene blue on the oxygen consumption of tumors and normal tissues // Journal of Experimental Medicine. – 1930. – Vol. 52. – № 3. – P. 447-456.
29. Sevcik P., Dunford H.B. Kinetics of the oxidation of NADH by methylene blue in a closed system // The Journal of Physical Chemistry. – 1991. – Vol. 95. – № 6. – P. 2411-2415.
30. Engbersen J.F.J., Koudijs A., Van Der Plas H.C. Reaction of NADH models with methylene blue // Recueil des Travaux Chimiques des Pays-Bas. – 2010. – Vol. 104. – № 5. – P. 131-138.
31. Schirmer R.H., Adler H., Pickhardt M., Mandelkow E. "Lest we forget you — methylene blue …" // Neurobiology of Aging. – 2011. – Vol. 32. – № 12. – P. 2325.e7-2325.e16.
32. Buchholz K., Schirmer R.H., Eubel J.K., Akoachere M.B., Dandekar T., Becker K., Gromer S. Interactions of Methylene Blue with Human Disulfide Reductases and Their Orthologues from Plasmodium falciparum // Antimicrobial Agents and Chemotherapy. – 2008. – Vol. 52. – № 1. – P. 183-191.
33. Komlódi T., Tretter L. Methylene blue stimulates substrate-level phosphorylation catalysed by succinyl–CoA ligase in the citric acid cycle // Neuropharmacology. – 2017. – Vol. 123. – P. 287-298.
34. Zhang H., Rogiers P., Preiser J.-C., Spapen H., Manikis P., Metz G., Vincent J.-L. Effects of methylene blue on oxygen availability and regional blood flow during endotoxic shock // Critical Care Medicine. – 1995. – Vol. 23. – № 10. – P. 1711-1721.
35. Tepaev R.F., Vishnevskiy V.A., Kuzin S.A., Sergey I.V., Gordeeva O.B., Pytal A.V., Murashkin N.N. Benzocaine-Induced Methemoglobinemia. A Clinical Case // Pediatric pharmacology. – 2018. – Vol. 15. – № 5. – P. 396-401.
36. Peter C., Hongwan D., Küpfer A., Lauterburg B.H. Pharmacokinetics and organ distribution of intravenous and oral methylene blue // European Journal of Clinical Pharmacology. – 2000. – Vol. 56. – № 3. – P. 247-250.
37. Lee S.-K., Mills A. Novel photochemistry of leuco-Methylene Blue // Chemical Communications. – 2003. – № 18. – P. 2366.
38. Stratonnikov A.A., Loschenov V.B. Evaluation of blood oxygen saturation in vivo from diffuse reflectance spectra // Journal of Biomedical Optics. – 2001. – Vol. 6. – № 4. – P. 457.
39. Sharick J.T., Favreau P.F., Gillette A.A., Sdao S.M., Merrins M.J., Skala M.C. Protein-bound NAD(P)H Lifetime is Sensitive to Multiple Fates of Glucose Carbon/ / Scientific Reports. – 2018. – Vol. 8. – № 1. – P. 5456.
40. Kalinina S., Freymueller C., Naskar N., Von Einem B., Reess K., Sroka R., Rueck A. Bioenergetic Alterations of Metabolic Redox Coenzymes as NADH, FAD and FMN by Means of Fluorescence Lifetime Imaging Techniques // International Journal of Molecular Sciences. – 2021. – Vol. 22. – № 11. – P. 5952.
41. Ranjit S., Malacrida L., Jameson D.M., Gratton E. Fit-free analysis of fluorescence lifetime imaging data using the phasor approach// Nature Protocols. – 2018. – Vol. 13. – № 9. – P. 1979-2004.
42. Worth K.R., Papandreou I., Hammond E.M. How the histological structure of some lung cancers shaped almost 70 years of radiobiology // British Journal of Cancer. – 2023. – Vol. 128. – № 3. – P. 407-412.

ORIGINAL ARTICLES

EFFECTIVENESS OF KATUK LEAF CHLOROPHYLL (SAUROPUS ANDROGYNUS (L) MERR) WITH BLUE AND RED LASER ACTIVATION TO REDUCE AGGREGATIBACTER ACTINOMYCETEMCOMITANS AND ENTEROCOCCUS FAECALIS BIOFILM

Permatasari P.A.D.[1], **Astuti S.D.**[1], **Yaqubi A.K.**[1], **Paisei E.A.W.**[1], **Pujiyanto**[1], **Nasrul Anuar**[2]
[1]Airlangga University, Surabaya, Indonesia
[2]Universiti Malaya, Kuala Lumpur, Malaysia

Abstract

In this study, the efficacy of using Sauropus androgynus (L) Merr, a katuk leaf chlorophyll photosensitizer, to reduce *Aggregatibacter actinomycetemcomitans* and *Enterococcus faecalis* biofilm was investigated. A red and blue diode laser is used as the light source. The sample was split into four groups: a negative control group, a positive control group, a blue laser treatment group (B), and a red laser treatment group (R), both with and without the addition of katuk leaf chlorophyll 1.6 mg/ml, and with varying densities of laser energy exposure of 2.5 J/cm^2, 5 J/cm^2, 7.5 J/cm^2, and 10 J/cm^2. Laser exposure and chlorophyll photosensitizer were tested using ELISA and ANOVA. At an energy density of 10 J/cm^2, the optimal bacterial mortality rate was obtained in each treatment group. Namely, in the *Aggregatibacter actinomycetemcomitans* biofilm, the negative group, the number of deaths was 73.30% using a blue diode laser and 63.25% using a red diode laser. In the positive group, the number of deaths was 86.12% using a blue diode laser and 83.29% using a red diode laser. In the *Enterococcus faecalis* biofilm, in the negative group, the number of deaths was 67.78% using the blue diode laser and 75.33% using the red diode laser, and in the positive group, the number of deaths was 71.71% using the blue diode laser and 86.41 using a red diode laser. Exposure to blue and red diode lasers activates chlorophyll in katuk leaves, killing bacteria and reducing biofilms.

Keywords: photoinactivation, blue and red diode laser, katuk leaf chlorophyll *(Sauropus androgynus (L) Merr)*, *Aggregatibacter actinomycetemcomitans*, *Enterococcus faecalis*.

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Contacts: Astuti S.D., e-mail: suryanidyah@fst.unair.ac.id

АНТИБАКТЕРИАЛЬНАЯ ЭФФЕКТИВНОСТЬ ХЛОРОФИЛЛА ЛИСТЬЕВ КАТУКА (SAUROPUS ANDROGYNUS (L) MERR) С АКТИВАЦИЕЙ СИНИМ И КРАСНЫМ ЛАЗЕРОМ В ОТНОШЕНИИ БИОПЛЕНКИ AGGREGATIBACTER ACTINOMYCETEMCOMITANS И ENTEROCOCCUS FAECALIS

P.A.D. Permatasari[1], **S. D. Astuti**[1], **A. K. Yaqubi**[1], **E.A.W. Paisei**[1], **Pujiyanto**[1], **Nasrul Anuar**[2]
[1]Airlangga University, Surabaya, Indonesia
[2]Universiti Malaya, Kuala Lumpur, Malaysia

Резюме

Изучена фотодинамическая активность фотосенсибилизатора хлорофилла листьев катука в отношении биопленки *Aggregatibacter actinomycetemcomitans* и *Enterococcus faecalis*. В качестве источника света был использован красный и синий диодный лазер. В исследование были четыре группы: группа отрицательного контроля, группа положительного контроля, группа обработки синим лазером (B) и группа обработки красным лазером (R), как с добавлением, так и без добавления хлорофилла листьев катука в концентрации 1,6 мг/мл, а также при различной плотности энергии лазерного излучения: 2,5 Дж/см^2, 5 Дж/см^2, 7,5 Дж/см^2 и 10 Дж/см^2. Эффективность воздействия оценивали с помощью ELISA и ANOVA. Наибольшая эффективность была зарегистрирована во всех режимах воздействия (красный/синий лазер, без/с хлорофиллом) при плотности энергии 10 Дж/см^2. В биопленке *Aggregatibacter actinomycetemcomitans* в контрольных группах (только облучение) эффективность составила 73,30% при использовании синего диодного лазера и 63,25% при использовании красного диодного лазера, а в опытных группах эффективность составила 86,12% при использовании синего диодного лазера и 83,29% при использовании красного диодного лазера. В биопленке *Enterococcus faecalis* в контрольных группах эффективность составила 67,78% при использовании синего диодного лазера и 75,33% при использовании красного диодного лазера, а в опытных группах эффективность составила 71,71% при использовании синего диодного лазера и 86,41% с использованием красного диодного лазера. Таким образом, сделан вывод, что воздействие синего и красного диодных лазеров активирует хлорофилл в листьях катука, обладая бактерицидным действием бактерии и уменьшая биопленки.

Ключевые слова: фотоинактивация, синий и красный диодный лазер, хлорофилл листьев катука (*Sauropus androgynus (L) Merr*), *Aggregatibacter actinomycetemcomitans*, *Enterococcus faecalis*.

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Контакты: Astuti S.D., e-mail: suryanidyah@fst.unair.ac.id

Introduction

In general, the bacteria *Aggregatibacter actinomycetemcomitans* and *Enterococcus faecalis* are to cause for dental and oral health issues. Due to a lack of public awareness about maintaining dental and oral hygiene, bacteria can form on the teeth and in the mouth. A gram-negative, facultative anaerobic coccobacillus that does not migrate is called *Aggregatibacter actinomycetemcomitans* (A.a.) [1]. One of the bacteria in the oral cavity that has the potential to induce periodontal disease, particularly localized aggressive periodontitis, is *Aggregatibacter actinomycetemcomitans* [2, 3]. The periodontal ligament and alveolar bone are damaged by periodontal disease caused by microorganisms [4, 5]. *Enterococcus faecalis* can form pockets in pairs, singletons, or short chains. E. faecalis is facultatively anaerobic and can cause root canal damage [6, 7].

Antibiotic overuse can lead to the development of biofilms, which have a defined structure, adhere to one another, and adhere to both living and inanimate objects [8]. As they grow, bacteria that make biofilms may be exposed to conditions that could kill them. Antibiotics, cleaning agents, and even the immune system of the host are all ineffective against the bacteria in the biofilm. Resistance to antibiotic therapy is the clinical symptom of a biofilm-forming bacterial infection [9]. The majority of bacteria in a biofilm will continue to live and proliferate, but only planktonic bacteria will be killed [10]. The photoinactivation method is effective and selective in eliminating *S. aureus* biofilm bacteria [11].

Free radicals, light, photosensitizers, PDI, and non-invasive photonics are therapeutic techniques [12]. The key to photoinactivation is photosensitization, which works by letting light in and setting off chemical reactions that make reactive oxygen species [13]. Lasers and LEDs are used to photoinactivate bacterial biofilms. Porphyrin compounds are light-sensitive photosensitizer molecules found in some bacteria. Photosensitizers are used to take in light energy, such as chlorophyll, which is used in photoinactivation therapy [14, 15]. The ability of chlorophyll to absorb light and transform it into energy is an implementation of the normal chlorophyll structure, which is primarily made up of porphyrins [16]. The comparatively lengthy (10–8 seconds) singlet chlorophyll excitation phase, which then passes through intersystem crossover to triplet excitation, is what results in the significant energy absorption of chlorophyll during photosynthesis. The closest oxygen molecule will receive the extra energy at the triplet excitation level to create reactive singlet oxygen [17].

Research using Dracaena angustifolia chlorophyll as a photosensitizing agent with a 405 nm blue laser led to an 80% reduction in S. aureus biofilm [18], 62% and 78% in S. mutant bacteria with pheophytin A and Alfalfa Medicago sativa L [19], and 22% and 60% on C. albican biofilm using 445 nm and 650 nm [20, 21].

Katuk leaves contain steroids and polyphenols, which increase prolactin levels and are anti-inflammatory and anti-diabetic. With a chlorophyll content of 1509.1 mg/kg [22], katuk leaves (*Sauropus androgynus (L) Merr*) serve as a model for a photosensitizer agent that is selective, effective, chemically stable, has a wide range of absorption wavelengths, is soluble, non-toxic, and non-toxic. In this work, blue and red diode laser light sources will be used to test the efficiency of katuk leaf chlorophyll (*Sauropus androgynus (L) Merr*) as an organic photosensitizing agent for inactivating *E. faecalis* and *A. acinomycetemcomitans* bacteria biofilms.

Materials and methods

Chlorophyll extraction of katuk leaves (Sauropus Androgynus (L) Merr) and antibacterial test

Using 96% ethanol, 50 grams of katuk leaves (*Sauropus androgynus (L) Merr*) were extracted. The recovered materials were then mixed with maltodextrin, which made up 20% of the filtrate's mass. The Shimidzu UV-Vis 1800 Spectrometer was used to evaluate the absorbance spectrum of the extracted materials. The disc diffusion method was then used to examine the antibacterial activity of chlorophyll on biofilm samples.

Diode laser characterization

A laser diode will be employed as the light source. Measurements of the wavelength spectrum, power stability, beam area, and temperature stability throughout treatment are all part of the light source's characterization. A temperature gun, a Thermolab PM-100 power meter, and a CT-100 wavelength meter are the instruments utilized for testing.

Bacterial culture and biofilm production

The samples were facultative anaerobe biofilms of the Gram-negative bacteria *Aggregatibacter actinomycetemcomitans* ATCC 29523 and the Gram-positive bacteria E. *faecalis* ATCC 29212. Bacterial cells were introduced to tryptic soy broth, a sterile agar medium, and 1 mL of 2% sucrose. The sample was then pipette-inserted into the microplate hole. Samples were incubated at 37 °C for 48 hours. For samples that have chlorophyll extract added, the extract will be applied before treatment, and the sample will be incubated for about two hours.

Treatment

Samples were distributed to the red laser treatment group (R) and the blue laser treatment group (B). Group B1 had the laser treatment group without the addition of chlorophyll, while group R1 was made up of each group. The katuk leaf chlorophyll (*Sauropus androgynus (L) Merr*) was also present at 1.6 mg/mL in the Groups B2 and R2 laser treatment groups. At a distance of 1 cm, a laser with energy densities ranging from 2.5 J/cm^2 to 10 J/cm^2 was utilized to treat each group. Each group has Group C_0, which stands for the negative control without the addition of chlorophyll or laser treatment, and Group C1, which stands for the treatment with chlorophyll but without laser. Laser exposure and chlorophyll photosensitizer were tested using ELISA and ANOVA to determine the effects of laser exposure and the addition of chlorophyll photosensitizer.

Results and discussion

Chlorophyll extraction of katuk leaves (Sauropus androgynus (L) Merr)

The findings of the absorbance test revealed that the chlorophyll absorbance peaks of katuk leaves were at wavelengths of 383 nm-419 nm and 500 nm-685 nm. The maximal absorbance of chlorophyll at 10% is 2.42. the following equation can be used to determine the amount of chlorophyll:

$$\text{Total chlorophyll} = [8{,}02(A663) + 20{,}20(A645)]\ \text{mg/L} \quad (1)$$

A 10% chlorophyll solution has 71.71 mg/L of total chlorophyll, according to equation 1. When employing diode laser light sources with wavelengths of 401 nm and 660 nm, 91% of the photosensitizer is absorbed. The results of the chlorophyll anti-bacterial test are shown in Table 1 for all concentration ranges, including control (0%), 2.5%, 5%, 7.5%, and 10%. The test findings revealed no clear zone on any of the disc papers, which suggests that the four concentrations are categorized as concentrations of compounds without antibacterial activity.

Diode laser characterization test results

Using a wavelength meter, a diode laser will be utilized as the light source. Its peak wavelengths are (401 ± 10) and (660 ± 7) nm, respectively (CT-100). On a blue diode laser (25.00 ± 1.71 mW) with a beam area of 0.20 cm and a red

Таблица 1
Диаметр зоны отсутствия роста в диско-диффузионном тесте
Table 1
The diameter of the clear zone in the disc diffusion test

Концентрация Concentration	Хлорофилл Chlorophyll	Диаметр зоны отсутствия роста Diameter of the clear zone			
		Бактерии Bacteria		Биопленка Biofilms	
		A.actinomycetemcomitans	*E. faecalis*	*A.actinomycetemcomitans*	*E. faecalis*
2,5%	50 µl	(0,00±0,05) cm	(0,00±0,05) cm	(0,00±0,05) cm	(0,00±0,05) cm
5%	50 µl	(0,00±0,05) cm	(0,00±0,05) cm	(0,00±0,05) cm	(0,00±0,05) cm
7,5%	50 µl	(0,00±0,05) cm	(0,00±0,05) cm	(0,00±0,05) cm	(0,00±0,05) cm
10%	50 µl	(0,00±0,05) cm	(0,00±0,05) cm	(0,00±0,05) cm	(0,00±0,05) cm

Таблица 2
Параметры облучения
Table 2
Irradiation parameters

Длина волны (нм) Wavelength (nm)	Мощность лазера (мВт) Laser power (mW)	Площадь зоны облучения (см²) Laser beam area (cm²)	Время (сек) Time (s)	Плотность энергии (Дж/см²) Energy density (J/cm²)
(401± 10)	(25.00 ± 1.71)	(0.20 ± 0.01)	20	2.51
			40	5.00
			60	7.50
			80	10.00
(660 ± 7)	(39.70 ± 1,35)	(0.24 ± 0,01)	15	2.50
			31	5.12
			45	7.50
			61	10.09

diode laser (39.7 ± 1.35 mW) with a beam area of (0.24 ± 0.01) cm, the power of the diode laser was measured under stable conditions using a Thermolab PM 100 power meter. The temperature was maintained at 37 °C throughout the irradiation. Equation 2 can be used to determine the two lasers' respective energy densities [23]. Table 2 displays the duration of the laser treatment.

$$E = \frac{P}{A} x t \qquad (2)$$

Inactivation photodynamic test results

Fig. 1 and 2 show what happens to *A. actinomycetemcomitans* and *E. faecalis* biofilms when they are exposed to radiation.

A biofilm caused by *A. actinomycetemcomitans* was able to survive after exposure to lasers and chlorophyll photosensitizer.

Fig. 3 and 4 show the percentage reduction of *A. actinomycetemcomitans and E. faecalis* biofilms, respectively.

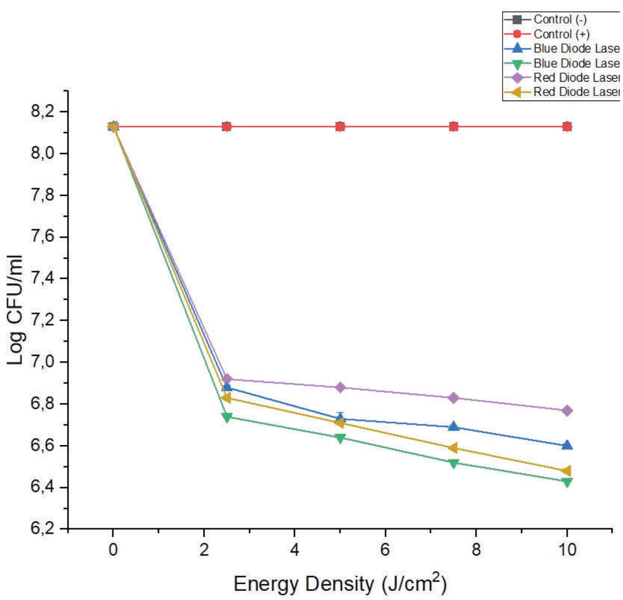

Рис. 1. Жизнеспособность биопленки *A. actinomycetemcomitans* при облучении с различной плотностью энергии с фотосенсибилизатором хлорофилл.
Fig. 1. *A. actinomycetemcomitans* biofilm viability in various laser treatments with variations in energy density and the addition of chlorophyll photosensitizer.

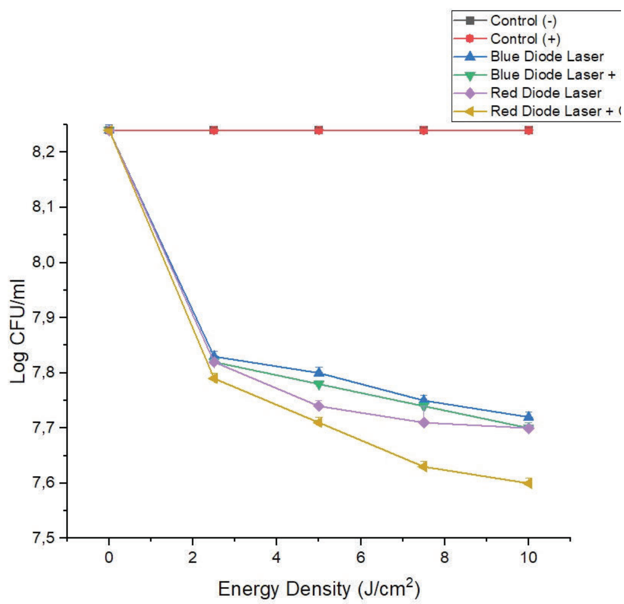

Рис. 2. Жизнеспособность биопленки *E. faecalis* при облучении с различной плотностью энергии с фотосенсибилизатором хлорофилл.
Fig. 2. *E. faecalis* biofilm viability in various laser treatments with variations in energy density and the addition of chlorophyll photosensitizer.

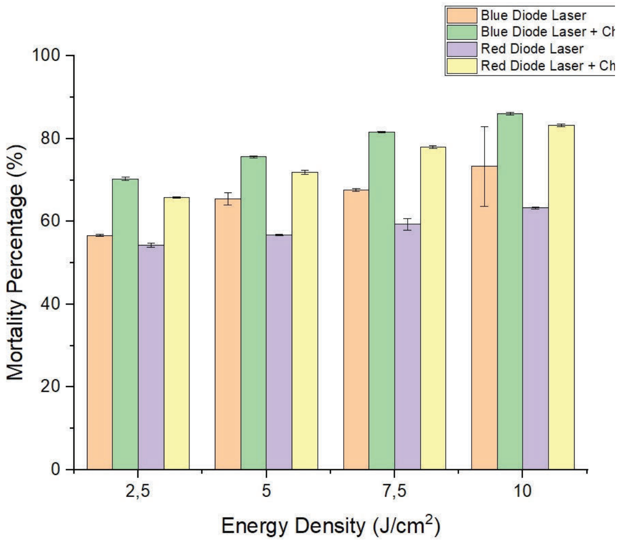

Рис. 3. Зависимость редукции биопленки *A. actinomycetemcomitans* от плотности энергии лазерного облучения.
Fig. 3. Dependence of *A. actinomycetemcomitans* biofilm reduction on laser irradiation energy density.

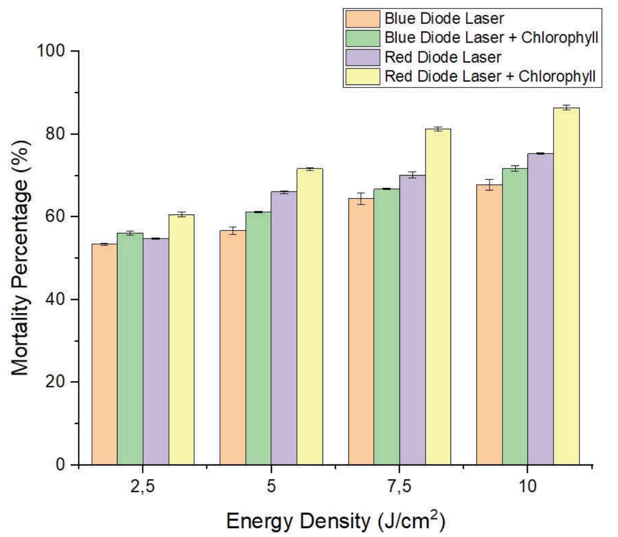

Рис. 4. Зависимость редукции биопленки *E. faecalis* в зависимости от плотности энергии лазерного облучения.
Fig. 4. Dependence of *E. faecalis* biofilm reduction on laser irradiation energy density.

The results of a factorial ANOVA test showed no significant differences between treatment groups ($p = 0$). The 10 J/cm^2 energy-dense blue diode laser treatment with 86.12% chlorophyll had the highest percentage of *A. actinomycetemcomitans* biofilm reduction, while the best decrease of *E. faecalis* biofilm was achieved with 2.5 J/cm^2.

Fluorescence test results

Testing with a fluorescent microscope to determine how many bacteria have died. Fig. 5 to 8 display the findings of the fluorescence test.

The 10 J/cm^2 energy-dense blue diode laser treatment group had the highest percentage of *A. actinomycetemcomitans* biofilm death, and the addition of chlorophyll caused 2122 cell deaths, according to the fluorescence test results. When chlorophyll was added to an energy of 10 J/cm^2, 2189 cell deaths occurred.

Chlorophyll produced by katuk leaves has no direct anti-bacterial activity. Chlorophyll becomes active as a photosensitizer exogen when exposed to the right spectrum. Triplet excitation creates radical oxygen species that render bacteria inactive due to cell membrane leakage. In this study, when blue and red diode lasers stimulated the chlorophyll in katuk leaves, radical oxygen species were made. These radical oxygen species killed bacteria and stopped *A. actinomycetemcomitan* and *E. faecalis* from making biofilms.

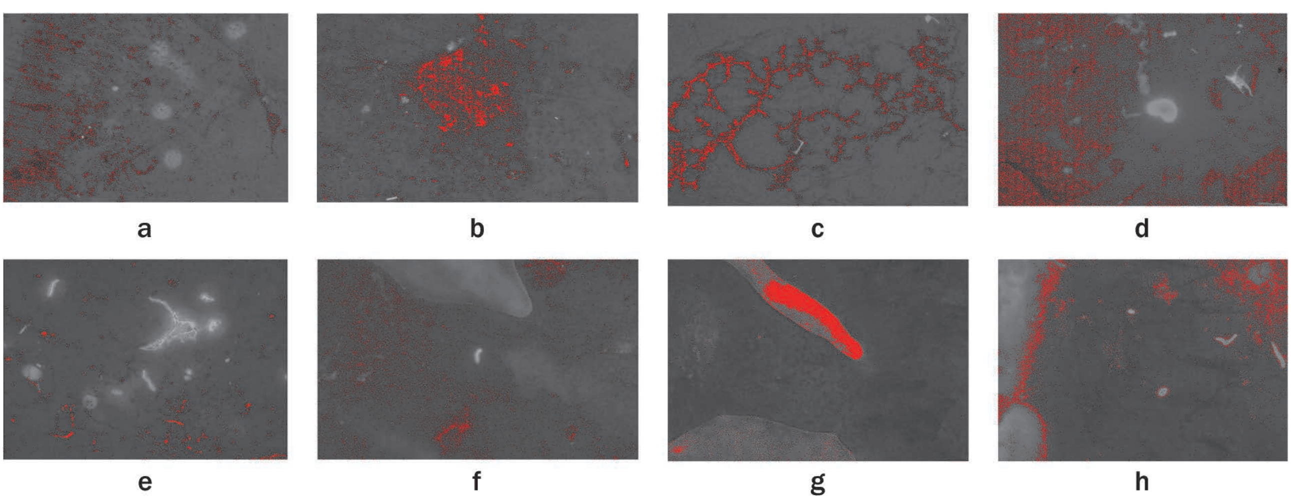

Рис. 5. Результаты воздействия на бактерии *A. actinomycetemcomitans* синим диодным лазером: a – 2,5 Дж/см^2; b – 5 Дж/см^2; c – 7,5 Дж/см^2, d – 10 Дж/см^2; и красным диодным лазером: e – 2,5 Дж/см^2; f – 5 Дж/см^2; g – 7,5 Дж/см^2; h – 10 Дж/см^2.
Fig. 5. Treatment results on *A. actinomycetemcomitans* bacteria with an energy-dense blue diode laser: a – 2.5 J/cm^2; b – 5 J/cm^2; c – 7.5 J/cm^2; d – 10 J/cm^2; and energy density red diode laser treatment: e – 2.5 J/cm^2; f – 5 J/cm^2; g – 7.5 J/cm^2; h – 10 J/cm^2.

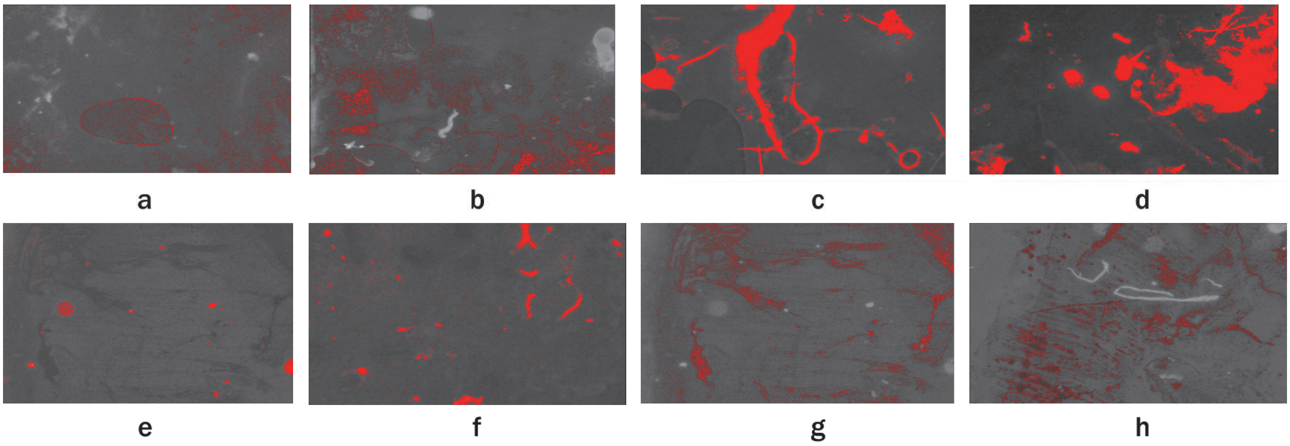

Рис. 6. Результаты воздействия на бактерии *A. actinomycetemcomitans* с добавлением хлорофилла синим диодным лазером: a – 2,5 Дж/см²; b – 5 Дж/см²; c – 7,5 Дж/см², d – 10 Дж/см²; и красным диодным лазером: e – 2,5 Дж/см²; f – 5 Дж/см²; g – 7,5 Дж/см²; h – 10 Дж/см².
Fig. 6. Treatment results on *A. actinomycetemcomitans* bacteria with the addition of chlorophyll with an energy-dense blue diode laser: a – 2.5 J/cm²; b – 5 J/cm²; c – 7.5 J/cm²; d – 10 J/cm²; and energy density red diode laser treatment: e – 2.5 J/cm²; f – 5 J/cm²; g – 7.5 J/cm²; h – 10 J/cm².

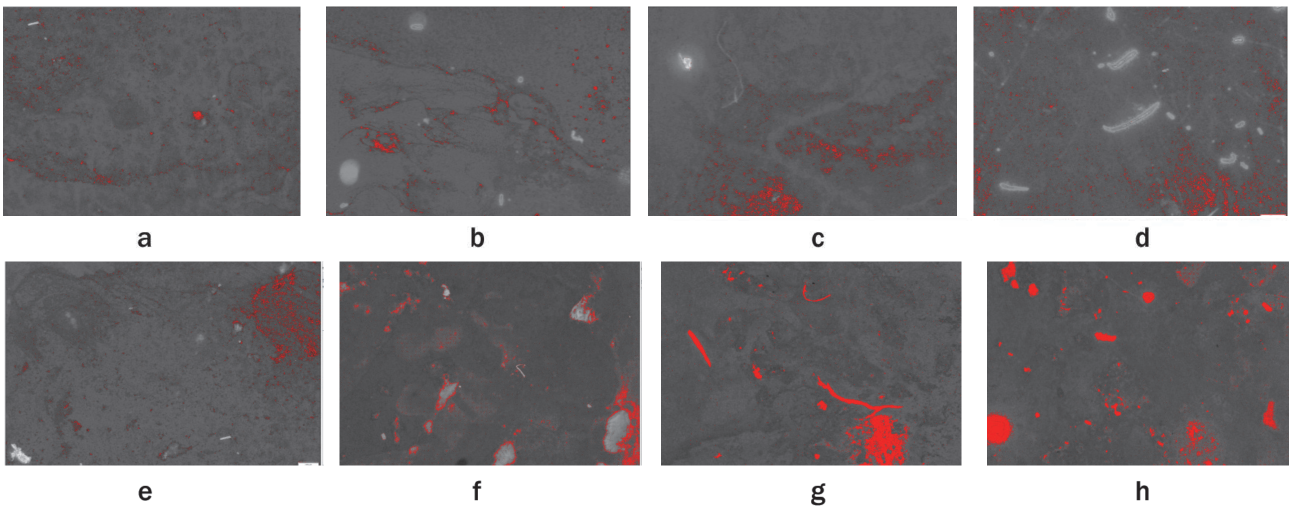

Рис. 7. Результаты воздействия на бактерии *E. faecalis* синим диодным лазером: a – 2,5 Дж/см²; b – 5 Дж/см²; c – 7,5 Дж/см², d – 10 Дж/см²; и красным диодным лазером: e – 2,5 Дж/см²; f – 5 Дж/см²; g – 7,5 Дж/см²; h – 10 Дж/см².
Fig. 7. The results of treatment on *E. faecalis* bacteria with an energy density blue diode laser: a – 2.5 J/cm²; b – 5 J/cm²; c – 7.5 J/cm²; d – 10 J/cm²; and energy density red diode laser treatment: e – 2.5 J/cm²; f – 5 J/cm²; g – 7.5 J/cm²; h – 10 J/cm².

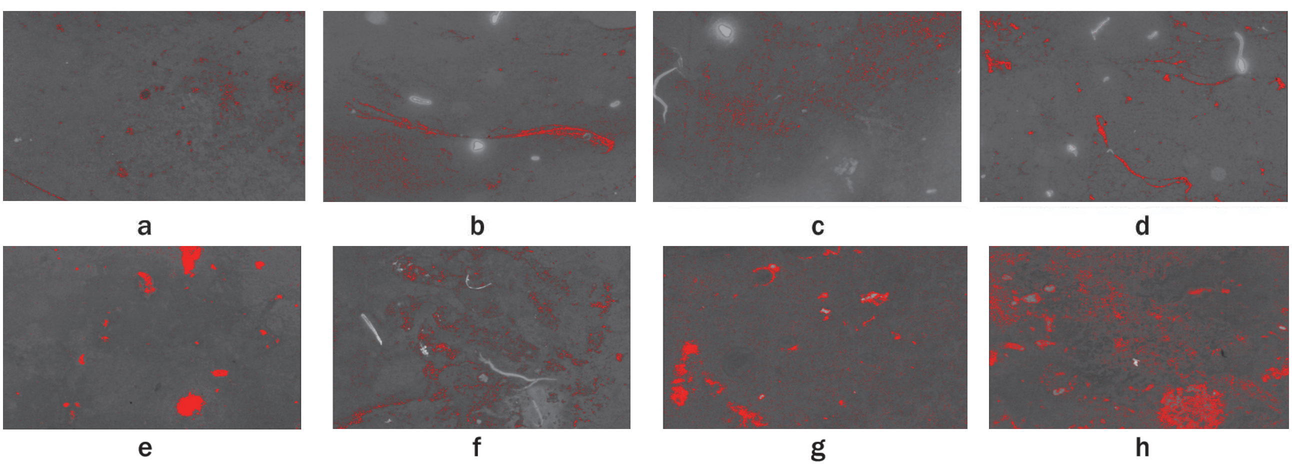

Рис. 8. Результаты воздействия на бактерии *E. faecalis* с добавлением хлорофилла синим диодным лазером: a – 2,5 Дж/см²; b – 5 Дж/см²; c – 7,5 Дж/см², d – 10 Дж/см²; и красным диодным лазером: e – 2,5 Дж/см²; f – 5 Дж/см²; g – 7,5 Дж/см²; h – 10 Дж/см².
Fig. 8. Treatment results on *E. faecalis* bacteria with the addition of chlorophyll with an energy density blue diode laser: a – 2.5 J/cm²; b – 5 J/cm²; c – 7.5 J/cm²; d – 10 J/cm²; and energy density red diode laser treatment: e – 2.5 J/cm²; f – 5 J/cm²; g – 7.5 J/cm²; h – 10 J/cm².

The *A. actinomycetemcomitans* biofilm was reduced by 86.12% in the 10 J/cm^2 energy-dense blue diode laser therapy with chlorophyll addition. The 2.5 J/cm^2 red diode laser treatment without the addition of chlorophyll had the largest reduction percentage of *A. actinomycetemcomitans* biofilm (54.34%). The energy-dense red diode laser treatment of 10 J/cm^2 with the addition of chlorophyll produced the highest percentage test results for the removal of *E. faecalis* biofilm, 86.41%. The best way to get rid of *E. faecalis* biofilm was to use a blue diode laser with a high energy density of 2.5 J/cm^2 without adding chlorophyll.

Red diode laser treatment of Gram-positive bacteria resulted in the greatest percentage of biofilm reduction. Gram-positive bacteria have fluorescence emission maxima at 622 nm and 617 nm, while gram-negative bacteria have emission peaks at 630 nm and 615 nm [24]. Gram-positive bacteria are more vulnerable to singlet oxygen, making chlorophyll more effective at killing them [25]. Gram-positive bacteria have a single layer of teichoic acid and a thick, porous peptidoglycan layer, while Gram-negative bacteria have two layers. Gram-negative bacteria are more sensitive to physical disturbances, with *E. faecalis* dying off at a higher rate than *A. actinomycetemcomitans*.

Conclusion

The 10 J/cm^2 energy-dense blue diode laser treatment with chlorophyll addition had the largest reduction percentage of *A. actinomycetemcomitans* biofilm, with a reduction percentage of 86.12%. Without the addition of chlorophyll, the red diode laser treatment reduced the *A. actinomycetemcomitans* biofilm by 54.34% at a density of 2.5 J/cm^2. While the red diode laser treatment with a 10 J/cm^2 energy density and the addition of chlorophyll had the greatest percentage reduction for *E. faecalis* biofilm (86.41%), the blue diode laser treatment with a 2.5 J/cm^2 energy density and the absence of chlorophyll had the greatest percentage reduction for *E. faecalis* biofilm (54.40%). So, when blue and red diode lasers hit katuk leaves, the chlorophyll gets turned on. This makes radical oxygen species, which kill bacteria and reduce *Aggregatibacter actinomycetemcomitans* and *E. faecalis* biofilms.

REFERENCES

1. Wibawa I.G.Y. et al. Aggregatibacter actinomycetemcomitans sensitivity towards chlorophyll of Moringa leaf after activated by diode laser. *Dental Journal*, 2016, Vol. 49(4), pp. 195-200
2. Setiawatie E.M. et al. Comparison of anti-bacterial efficacy of photodynamic therapy and doxycycline on aggregatibacter actinomycetemcomitans. *Afr. J. Infect. Dis*, 2018, Vol. 12(5), pp. 95-103
3. Setiawatie E.M. et al. An in vitro Anti-microbial Photodynamic Therapy (aPDT) with Blue LEDs to Activate Chlorophylls of Alfalfa Medicago sativa L on Aggregatibacter actinomycetemcomitans. *J. Int. Dent. Med. Res*, 2016, Vol. 9(2), pp. 111-117
4. Hajishengallis G and Chavakis T. Local and systemic mechanisms linking periodontal disease and inflammatory comorbidities. *Nature Reviews Immunology*, 2021,Vol. 21(7), pp. 426-440
5. Astuti S.D. et al. Combination effect of laser diode for photodynamic therapy with doxycycline on a wistar rat model of periodontitis. *BMC Oral Health*, 2021, Vol. 21(1), pp. 80. doi: 10.1186/s12903-021-01435-0
6. Najafi K. et al. Oral cavity infection by Enterococcus faecalis: virulence factors and pathogenesis. *Reviews and Research in Medical Microbiology*, 2020, Vol. 31(2), pp. 51-60
7. Alghamdi F and Shakir M. The influence of Enterococcus faecalis as a dental root canal pathogen on endodontic treatment. *A systematic review. Cureus*, 2020, Vol. 12(3), pp. e7257. doi: 10.7759/cureus.7257
8. Astuti S.D. et al. Effectiveness of Bacterial Biofilms Photodynamic Inactivation Mediated by Curcumin Extract, Nanodoxycycline and Laser Diode. *Biomedical Photonics*, 2020, Vol. 9(4), pp. 4-14. doi.org/10.24931/2413-9432-2020-9-4-4-14
9. Mah T. F. Biofilm-specific antibiotic resistance. *Future microbiology*, 2012, Vol. 7(9), pp. 1061-1072
10. Høiby N. et al. Antibiotic resistance of bacterial biofilms. *International journal of antimicrobial agents*, 2010, Vol. 35(4), pp. 322-332
11. Astuti S.D. et al. The efficacy of photodynamic inactivation with laser diode on Staphylococcus aureus biofilm with various ages of biofilm. *Infectious Disease Reports*, 2020, Vol. 12(18736), pp. 68-74
12. Almeida A. et al. Photodynamic inactivation of bacteria: finding the effective targets. *Future medicinal chemistry*, 2015, Vol. 7(10), pp. 1221-1224

ЛИТЕРАТУРА

1. Wibawa I.G.Y. et al. Aggregatibacter actinomycetemcomitans sensitivity towards chlorophyll of Moringa leaf after activated by diode laser // Dental Journal. – 2016. – Vol. 49(4). – P. 195-200
2. Setiawatie E.M. et al. Comparison of anti-bacterial efficacy of photodynamic therapy and doxycycline on aggregatibacter actinomycetemcomitans // Afr. J. Infect. Dis. – 2018. – Vol. 12(5). – P. 95-103
3. Setiawatie E.M. et al. An in vitro Anti-microbial Photodynamic Therapy (aPDT) with Blue LEDs to Activate Chlorophylls of Alfalfa Medicago sativa L on Aggregatibacter actinomycetemcomitans // J. Int. Dent. Med. Res. – 2016. – Vol. 9(2). – P. 111-117
4. Hajishengallis G and Chavakis T. Local and systemic mechanisms linking periodontal disease and inflammatory comorbidities // Nature Reviews Immunology. – 2021. –Vol. 21(7). – P 426-440
5. Astuti S.D. et al. Combination effect of laser diode for photodynamic therapy with doxycycline on a wistar rat model of periodontitis // BMC Oral Health. – 2021. – Vol. 21(1). – P. 80. doi: 10.1186/s12903-021-01435-0
6. Najafi K. et al. Oral cavity infection by Enterococcus faecalis: virulence factors and pathogenesis // Reviews and Research in Medical Microbiology. – 2020. – Vol. 31(2). – P. 51-60
7. Alghamdi F and Shakir M. The influence of Enterococcus faecalis as a dental root canal pathogen on endodontic treatment // A systematic review. Cureus. – 2020. – Vol. 12(3). – P. e7257. doi: 10.7759/cureus.7257
8. Astuti S.D. et al. Effectiveness of Bacterial Biofilms Photodynamic Inactivation Mediated by Curcumin Extract, Nanodoxycycline and Laser Diode // Biomedical Photonics. – 2020. – Vol. 9(4). – P. 4-14. doi.org/10.24931/2413-9432-2020-9-4-4-14
9. Mah T. F. Biofilm-specific antibiotic resistance // Future microbiology. – 2012. – Vol. 7(9). – P. 1061-1072
10. Høiby N. et al. Antibiotic resistance of bacterial biofilms // International journal of antimicrobial agents. – 2010. – Vol. 35(4). – P. 322-332
11. Astuti S.D. et al. The efficacy of photodynamic inactivation with laser diode on Staphylococcus aureus biofilm with various ages of biofilm // Infectious Disease Reports. – 2020. – Vol. 12(18736). – P. 68-74
12. Almeida A. et al. Photodynamic inactivation of bacteria: finding the effective targets // Future medicinal chemistry. – 2015. – Vol. 7(10). – P. 1221-1224

13. Verhille M. et al. Modulation of photosensitization processes for an improved targeted photodynamic therapy. *Current medicinal chemistry*, 2010, Vol. 17(32), pp. 3925-3943
14. Mardianto A.I. et al. Photodynamic Potential of Blue Diode Laser Inactivation with Chlorophyll Photosensitisers in Pseudomonas aeruginosa and Staphylococcus aureus bacteria, Proc. SPIE 11789. *Fourth International Seminar on Photonics, Optics, and Its Applications ISPhOA*, 2020, Vol. 117890J, pp. 11789. doi.org/10.1117/12.2587052
15. Uliana M. P. et al. Photobiological characteristics of chlorophyll a derivatives as microbial PDT agent. *Photochemical & Photobiological Sciences*, 2014, Vol. 13, pp. 1137-1145
16. Perez-Galvez A. et al. Chemistry in the bioactivity of chlorophylls: An overview. *Current medicinal chemistry*, 2017, Vol. 24(40), pp. 4515-4536
17. Astuti S.D. et al. Efficacy of CNC-diode laser combine with chlorophylls to eliminate Staphylococcus aureus biofilm. *Publishing in International Seminar Sensors, Instrumentation, Measurement, and Metrology (ISSIMM), IEEE Xplore*, 2017, pp. 57-61. doi.org/10.1109/ISSIMM.2016.7803722
18. Astuti S.D. et al. Photodynamic effectiveness of laser diode combined with ozone to reduce Staphylococcus aureus biofilm with exogenous chlorophyll of Dracaena angustifolia leaves. *Biomedical Photonic*, 2019, Vol. 8(2), pp. 4-13. doi.org/10.24931/2413-9432-2019-8-2-4-13
19. Sunarko S. A. et al. antimicrobial effect of pleomeleangustifolia pheophytin A activation with diode laser to streptococcus mutans. *Journal of Physics: Conference Series*, 2017, Vol. 853(1), pp. 012039. IOP Publishing.
20. Astuti S.D. et al. Chlorophyll mediated photodynamic inactivation of blue laser on Streptococcus mutans. *AIP Conference Proceedings*, 2016, Vol. 1718, pp. 120001
21. Astuty S.D. et al. The Efficacy of Photodynamic Inactivation of the Diode Laser in Inactivation of the Candida albicans Biofilms with Exogenous Photosensitizer of Papaya Leaf Chlorophyll. *J Lasers Med Sci*, 2019, Vol. 10(3), pp. 215-224
22. Nurdin, N. et al. Chlorophyll content of various types of plant leaves and Cu-chlorophyll derivatives and their physico-chemical characteristics. *Journal of Nutrition and Food*, 2009, Vol. 4(1), pp. 13-19
23. Yaqubi A.K. et al. Effectiveness of purple led for inactivation of Bacillus subtilis and Escherichia coli bacteria in in vitro sterilizers. *Biomedical Photonics*, 2022, Vol. 11(4). pp. 4-10. doi.org/10.24931/2413-9432-2022-11-4-4-10
24. Nitzan, Y. et al. ALA induced photodynamic effects on gram positive and negative bacteria. *Photochemical & Photobiological Sciences*, 2004, Vol. 3(5), pp. 430-435.
25. Huang L. et al. Type I and Type II mechanisms of antimicrobial photodynamic therapy: an in vitro study on gram-negative and gram-positive bacteria. *Lasers in surgery and medicine*, 2012, Vol. 44(6), pp. 490-499.

13. Verhille M. et al. Modulation of photosensitization processes for an improved targeted photodynamic therapy // Current medicinal chemistry. – 2010. – Vol. 17(32). – P. 3925-3943
14. Mardianto A.I. et al. Photodynamic Potential of Blue Diode Laser Inactivation with Chlorophyll Photosensitisers in Pseudomonas aeruginosa and Staphylococcus aureus bacteria, Proc. SPIE 11789 // Fourth International Seminar on Photonics, Optics, and Its Applications ISPhOA. – 2020. – Vol. 117890J. – P. 11789. doi.org/10.1117/12.2587052
15. Uliana M. P. et al. Photobiological characteristics of chlorophyll a derivatives as microbial PDT agent // Photochemical & Photobiological Sciences. – 2014. – Vol. 13. – P. 1137-1145
16. Perez-Galvez A. et al. Chemistry in the bioactivity of chlorophylls: An overview. Current medicinal chemistry. – 2017. – Vol. 24(40). – P. 4515-4536
17. Astuti S.D. et al. Efficacy of CNC-diode laser combine with chlorophylls to eliminate Staphylococcus aureus biofilm // Publishing in International Seminar Sensors, Instrumentation, Measurement, and Metrology (ISSIMM), IEEE Xplore. – 2017. – P. 57-61. doi.org/10. 1109/ISSIMM.2016.7803722
18. Astuti S.D. et al. Photodynamic effectiveness of laser diode combined with ozone to reduce Staphylococcus aureus biofilm with exogenous chlorophyll of Dracaena angustifolia leaves // Biomedical Photonic. – 2019. – Vol. 8(2). – P. 4-13. doi.org/10.24931/2413-9432-2019-8-2-4-13
19. Sunarko S. A. et al. antimicrobial effect of pleomeleangustifolia pheophytin A activation with diode laser to streptococcus mutans // Journal of Physics: Conference Series. – 2017. – Vol. 853(1). – P. 012039. IOP Publishing.
20. Astuti S.D. et al. Chlorophyll mediated photodynamic inactivation of blue laser on Streptococcus mutans // AIP Conference Proceedings. – 2016. – Vol. 1718. – P. 120001
21. Astuty S.D. et al. The Efficacy of Photodynamic Inactivation of the Diode Laser in Inactivation of the Candida albicans Biofilms with Exogenous Photosensitizer of Papaya Leaf Chlorophyll // J Lasers Med Sci. – 2019. – Vol. 10(3). – P. 215-224
22. Nurdin, N. et al. Chlorophyll content of various types of plant leaves and Cu-chlorophyll derivatives and their physico-chemical characteristics // Journal of Nutrition and Food. – 2009. – Vol. 4(1). – P. 13-19
23. Yaqubi A.K. et al. Effectiveness of purple led for inactivation of Bacillus subtilis and Escherichia coli bacteria in in vitro sterilizers // Biomedical Photonics. – 2022. – Vol. 11(4). – P. 4-10. doi.org/10.24931/2413-9432-2022-11-4-4-10
24. Nitzan, Y. et al. ALA induced photodynamic effects on gram positive and negative bacteria // Photochemical & Photobiological Sciences. – 2004. – Vol. 3(5). – P. 430-435.
25. Huang L. et al. Type I and Type II mechanisms of antimicrobial photodynamic therapy: an in vitro study on gram-negative and gram-positive bacteria // Lasers in surgery and medicine. – 2012. – Vol. 44(6). – P. 490-499.

ORIGINAL ARTICLES

THE BACTERICIDAL EFFECTS OF 632.8 NM HE-NE LASER ON STAPHYLOCOCCUS AUREUS COLONIES

Salih W.H.[1], Hassan S.H.[2]
[1]University of Technology and Applied Sciences, Suhar, Oman
[2]Al Neelain University, Khartoum, Sudan

Abstract

The bactericidal effect of 632.8 nm low level laser has been studied in order to point out both the effective power and laser exposure time on *Staphylococcus aureus*, which is reported to be involved in several dermatology problems. Low level laser has been reported to be useful for infected wounds, tissue necrosis, nerve injury, osteoarthritis or other chronic pain syndromes. Numerous studies have been conducted to quantify the effective laser parameters, i.e. dose, power, and exposure time, which ultimately leads toward clinical implementation. *Staphylococcus aureus* bacteria colonies were exposed to laser doses with powers of both 1 and 3 mW at different exposure times varies between 3 to 30 minutes. The bacterial colonies were isolated from a patient with inflamed wounds. Two sets of bacterial colonies were prepared to be exposed to laser beam. Next, the bacterial colonies were compared before and after exposing them to laser doses. The results showed that laser sessions have reduced the number of the bacterial colonies for both doses; 1 and 3 mw at the different exposure times and concentrations. The results revealed significant dose dependent bactericidal effects of He-Ne laser on *Staphylococcus aureus* at 3 mW for 30 minutes, which was found to be more effective in reducing the amount of bacteria to the less than 2% of its initial count. The results exhibited the reduction of the number of colonies as a function of exposure time. Appropriate doses of 632.8 nm can kill *Staphylococcus aureus*, suggesting that a similar effect may be used in clinical cases of bacterial infection.

Keywords: *Staphylococcus aureus*, low level laser therapy, laser exposure.

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Contacts: Salih W.H., e-mail: wasil.soh@cas.edu.om

БАКТЕРИЦИДНЫЙ ЭФФЕКТ HE-NE ЛАЗЕРА (632,8 НМ) НА КОЛОНИИ STAPHYLOCOCCUS AUREUS

Salih W.H.[1], Hassan S.H.[2]
[1]University of Technology and Applied Sciences, Suhar, Oman
[2]Al Neelain University, Khartoum, Sudan

Резюме

Нами был изучен бактерицидный эффект низкочастотного лазера с длиной волны 632,8 нм с целью определения эффективной мощности и времени воздействия лазера на бактерии *Staphylococcus aureus*, участвующие в патогенезе ряда дерматологических заболеваний. Ранее проведены многочисленные исследования количественной оценки эффективных параметров лазера: световой дозы, плотности мощности и времени воздействия. В настоящем исследовании на колонии бактерий *Staphylococcus aureus* воздействовали лазерным излучением мощностью 1 и 3 мВт при разном времени воздействия (от 3 до 30 мин). Колонии бактерий были выделены у больного с воспаленными ранами. Воздействие лазером уменьшило количество бактериальных колоний во всех экспериментах. Результаты выявили значительное дозозависимое бактерицидное воздействие гелий-неонового лазера на *Staphylococcus aureus.* При мощности 3 мВт при воздействии в течение 30 мин количество бактерий снизилось до уровня менее 2% от его первоначального количества. Результаты показали уменьшение количества колоний в зависимости от времени воздействия. Лазерное излучение на длине волны 632,8 нм обладает бактерицидным действием в отношении *Staphylococcus aureus*.

Ключевые слова: *Staphylococcus aureus*, низкоинтенсивная лазерная терапия, воздействие лазером.

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Контакты: Salih W.H., e-mail: wasil.soh@cas.edu.om

Introduction

Light therapy has been suggested as a potentially effective medical treatment approach for a variety of human conditions. Suggested amenable conditions range from sleep disorders, photo-aged facial skin, depression in the elderly, and treatment of acne *vulgaris* to a variety of neuron-musculoskeletal conditions such as peripheral neuropathy, second degree ankle sprains, and osteoarthritis of the knee and cervical spin [1,2,3,4]. Therefore, the use of low level laser therapy (LLLT) has become wide-spread in medicine [5,6,7].

LLLT is, mostly, used in clinical practice for the promotion of tissue healing and pain control. Specific applications of laser are diverse, in part, because each of the involved mechanisms can be applied to a number of body systems. Examples include management of indolent or infected wounds, tissue necrosis due to envenomation, nerve injury, osteoarthritis or other chronic pain syndromes, fracture healing, tendinous or ligamentous injury, and post-surgical incision care [1,5,8]. Nevertheless, laser treatments that are intended to stimulate protein synthesis in wounds may also reduce the bacterial growth, which would further stimulate the wound healing [9]. It's known that the bacterial infection is the most common problems underlying chronic wounds, therefore, there are limited options for the management of infected wounds and bacterial colonies, and some commonly used methods have undesired side effects. For example, typical disinfectants, such as hydrogen peroxide, that could eliminate certain bacteria as well as being toxic to new granulation tissue. The development of antibiotic resistant strains of bacteria is a limiting factor in the prophylactic use of broad spectrum antibiotics. Low-intensity laser therapy (LILT) has been investigated as a bio-stimulatory modality for the treatment of killing bacteria [5,10].

In the literature, three are several reports claimed that LLLT/LILT can facilitate the healing processes of many disorders. However, there is still significant debate regarding the efficiency of laser in producing the desired clinical response [8]. Among such studies, Ribeiro et al. (2004) have investigated the influence of low intensity polarized visible laser radiation on the acceleration of skin wound healing. Their histological analysis showed that the healing of irradiated wounds was faster than that of non-irradiated wounds. Moreover, it was observed that skin wound repair is dependent on polarization orientation with respect to a referential axis [11].

Another field in which LLLT was used is the bactericidal effects of laser. Nussbaum et al. (2002) have studied the effects of 630, 660, 810, and 905 nm laser irradiation at delivering radiant exposure of 1-50 J/cm^2 on three species of bacteria (*S. aureus* (ATCC 29213), *E. coli* (ATCC 25299), and *P. aeruginosa* (ATCC 27853)). They found that the applied LLLT to wounds, with radiant exposures in the range of 1-20 J/cm^2, could produce changes in bacterial growth of considerable importance for wound healing. A wavelength of 632.8 nm appeared to be the most commonly associated with bacterial inhibition. Their findings might be useful as a basis for selecting LLLT for infected wounds [5]. In addition, Guffey & Wilborn (2006) reported some bactericidal effects of 405 and 470 nm light on two bacteria: *S. aureus* and *P. aeruginosa.* Their results indicated that, *in vitro*, 405 and 470 nm blue light produce dose dependent bactericidal effects *S. aureus* and *P. aeruginosa* but not on *P. acnes* [1].

Moreover, LLLT finds its way to dentistry applications. In this concern, Folwaczny et al. (2002) have studied the antimicrobial effects of Er: YAG laser radiation on teeth root surfaces. Depending on the number of laser pulses, the bacterial load in the *E. coli* group has been reduced by the Er: YAG laser radiation after exposure to 105 laser pulses to 5.5% of the initial count, while the *S. aureus* group was reduced to 15.1% of the initial count. Beside the selective removal of plaque and calculus, the Er: YAG laser radiation causes reduction in bacteria on root surfaces [12]. However, the *S. aureus* has been reported to be killed via He-Ne laser pulses, even for the methicillin-resistant *S. aureus* (MRSA) [12,13].

On the other hand, Avram & Rogers (2009) have tried to solve hair health problems, such as hair growth through LLLT. Their results indicated that, on average, patients had a decrease in the number of vellums hairs, an increase in the number of terminal hairs, and an increase in shaft diameter when they were exposed to laser pulses. However, their results showed some limitations, since some of their findings were not statistically significant [14]. In addition, Avaci et al. (2014) have used LLLT to stimulate the hair growth in mice, which were subjected to chemotherapy induced alopecia and also in alopecia aerate. Among various mechanisms, the main mechanism is hypothesized to be stimulation of epidermal stem cells in the hair follicle bulge and shifting the follicles into anlagen phase [15].

In regards to the laser doses, the treatment dose is calculated as the amount of energy (Watts) delivered over a period of time or a specific tissue area. However, doses are often listed as Watts (Joules per second) per square centimetre or Joules (intensity of the energy in Watts multiplied by the treatment time) per square centimetre. Thus, the energy emitted per unit of time, total energy administered, the size of the area being treated and the treatment time are all important and interrelated variables in determining the desired laser doses [8]. Nevertheless, laser light is emitted either in a continuous wave (CW) or pulsed form. When laser light is emitted in pulses, the pulse frequency may impact the effectiveness of the treatment. Other variables that impact the effectiveness of the treatment include the distance from the laser source to the tissue surface and the target tissue, the speed of movement over the treatment area, and the number and frequency of treatments [8]. In conclusion, the physiologic effects of laser are reported to include stimulation of mitochondrial activity, increased cell turnover, recruitment and proliferation, modulation of the cellular metabolites involved in the inflammatory response, vasodilatation, involved in the inflammatory response, vasodilatation, release of exogenous endorphins, and increased oxygen availability in the tissues [10,16,17].

From the previously mentioned studies, the He-Ne laser seems to have antimicrobial properties, with the ability to kill a wide range of bacteria including *E coli, P. aeruginosa* and *S. aureus*, which is reported to be involved in several dermatology problems [18,19,20].

Therefore, one could propose that LLLT presents a great opportunity in treating bacteria related diseases. In this context, the current study is conducted to point out the possibility of using laser therapy to reduce the *S. aureus* count, as well as reporting the effective laser parameters to achieve that.

Materials and methods

For this study, a bacterium sample has been collected from a patient with an injury with inflammation, and then cultured in media to get a pure culture for a specific type of bacteria. The samples collection and processing were conducted according to the local ethical committee of Al Neelian University, the Sudan, and were in accordance to the International Guiding Principles for Research Involving Animals and Human Beings. The media for Mannitol Salt Agar (MSA) have been taken in a flask and dissolved in distilled water; the type of this medium was used as selective media for the desired bacteria, *S. aureus*. The solution was transferred to autoclave in order to make it sterilized. The autoclave was used with a pressure of 5 Pascal, under temperature of 121ºC for 15 minutes. Next, the media have been put on sterilized plates, and then the bacteria were cultured by a sterile loop in plates and incubated for 24 hours at 37 ºC. After the growth of the bacteria, they were a subject for further tests to be classified as *S. aureus* using H_2O_2 test. Samples from colonies have been taken and placed in a tube then the bacteria suspension was prepared. In order to do that, 1.5 g of Peptone water, which was measured by sensitive balance, were added to 0.2 g of distilled water. The mixture was subjected to sterilization by autoclave. Next, 10 ml was drained in a tube which contains Peptone water. Bacteria have been taken by loop and cultured in tube contained Peptone water.

Seven Eppendorf tubes in separate step were sterilized. Each one of the tubes contained 1 ml from the bacterial suspension. The next step was to expose the tubes to He-Ne 632.8 nm laser with a power of 1 mw for several time intervals: 3, 6, 9, 12, 15 and 18 minutes. After exposure to the laser beam, directly the samples drained and cultured in 6 plates contain MSA media. The flame used as sterilizer during the process culture. However, one tube was used as a control, thus it has never been subject to laser exposure. All of the plates involved in this study were incubated for 24 hours, including the control plate. After incubation, the growth colonies on each plate were counted. The experiment was repeated to check for the concentration of the suspension, which was an important issue to estimate the bacterial growth after exposing to laser doses.

In the dilution process, 6 empty tubes were used to make a serial dilution from bacterial suspension. 0.1 ml has been taken from the suspension by micro pipette and drained in the first tube. The sample was shaken and added to 0.9 ml of distilled water. Then, 0.1 ml of this mixture was taken from the tube (numbered as tube number one) using micro pipettes and placed in another tube, labelled as the tube number two that contained 0.9 ml distilled water. This step was repeated for four times in order to prepare six tubes. From each one of the previous tubes, 0.1 ml of the solution was taken and cultured in MSA media. After incubation for 24 hours, the bacteria were grown on the plates and created colonies.

Colony counter was used to count the number of colonies on each plate. The tube with bacterial

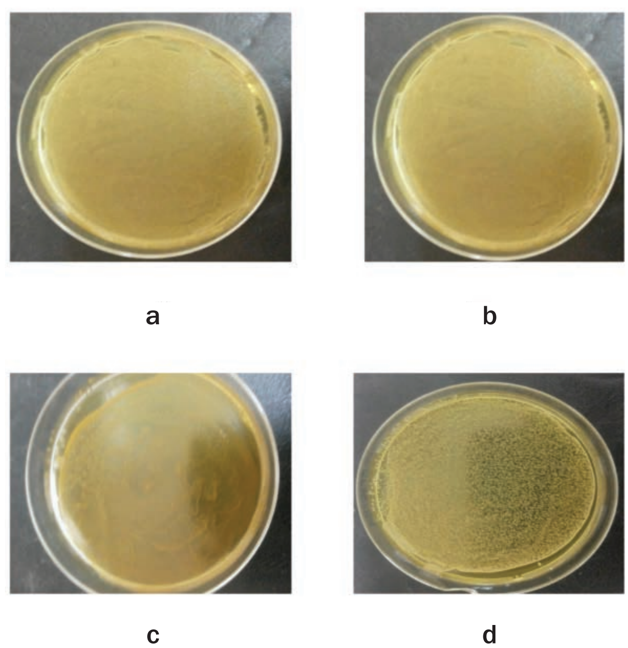

Рис. 1. Колонии *Staphylococcus aureus*, проба №A1:
a – контрольный образец; b – колонии после воздействия лазером в течение 3 мин; c – колонии после воздействия лазером в течение 6 мин; d – колонии после облучения лазером в течение 18 мин.

Fig. 1. Snapshots of the *Staphylococcus aureus* plates of the sample #A1: a – control sample; b – colonies after exposing to laser for 3 minutes; c –colonies after exposing to laser for 6 minutes; d – colonies after irradiation to laser for 18 minutes.

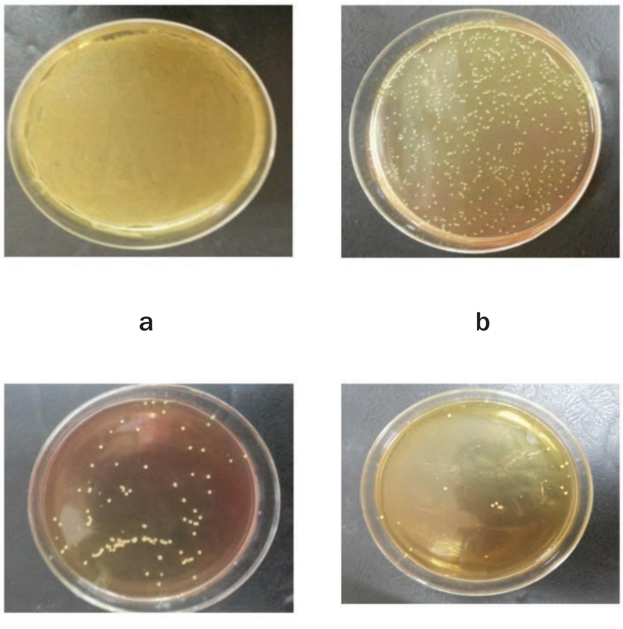

Рис. 2. Колонии *Staphylococcus aureus*, проба №А2: а – контрольный образец; b – колонии после воздействия лазером в течение 5 мин; с – колонии после воздействия лазером в течение 15 мин; d – колонии после облучения лазером в течение 30 мин.
Fig. 2. Snapshots of the *Staphylococcus aureus* plates of the sample #A2: a – control sample; b – colonies after exposing to laser for 5 minutes; c –colonies after exposing to laser for 15 minutes; d – colonies after irradiation to laser for 30 minutes.

suspension of higher concentration have been selected for further study, of which the tubes were exposed to He-Ne 632.8 nm laser, using KZ-350-LB setup, at output power of 3 mW with different exposure times (5, 10, 15, 20, 25 and 30 minutes). The number of colonies after exposing to laser in each time interval was counted in order to check for the decrease in the bacteria as a function of exposing to laser.

Results and discussion

The numbers of colonies of the *S. aureus* bacteria in the Eppendorf tubes have been counted both before and after irradiating the bacteria to the laser. This way, one sees whether they are affected by laser irradiation, and the effective exposure time that helps to reduce the bacteria to the minimum. Fig. 1a shows the colonies in the control sample while Fig. 1d shows the bacteria after laser exposure for 18 minutes. The same for sample №A2 is presented in Fig. 2. Fig. 2a shows the control sample while Fig. 2d shows the colonies after 30 minutes of laser irradiation.

Table represents the number of the bacterial colonies that counted for sample №A2 every 5 minutes of exposing to the laser, till almost bacteria are reduced to the minimum. A plot of those results is presented in Fig. 3.

Таблица
Количество колоний бактерий при разном времени экспозиции для образца №А2
Table
The bacteria colonies number as a function of exposure time for sample #A2

Время экспозиции (мин) Exposure time (minutes)	Число колоний Number of colonies
0	500 ± 25
5	400 ± 22
10	220 ± 14
15	175 ± 8
20	97 ± 5
25	25 ± 3
30	10 ± 2

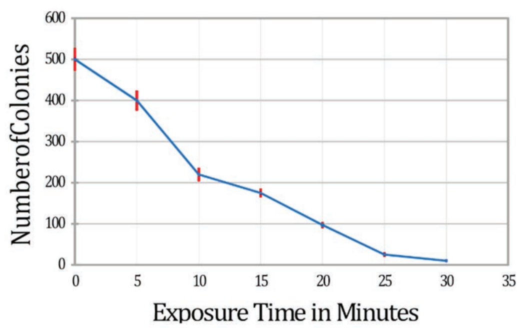

Рис. 3. Зависимость количества колоний бактерий от времени лазерного воздействия.
Fig. 3. The number of bacterial colonies as a function of the laser exposure time.

In this study, two *S. aureus* bacteria samples were exposed to a He-Ne laser source with a wavelength of 632.8 nm laser irradiation. The first one has been exposed to a power of 1 mW for different exposure times; 3, 6, 9, 12, 15 and 18 minutes. However, for the second sample, 3 mW was used at exposure times of 5, 10, 15, 20, 25 and 30 minutes. This way, one can see the effective laser parameters that could be used to reduce the bacteria to the minimum.

From the experiments, one notices that the number of bacterium colonies is decreased gradually as a function of exposure time, mainly at a laser power of 3 mW. The reduction in the colonies number occurs due to some effects and changes that laser is involved in. These effects were reported to include making holes or pores on the bacterial cells wall when they were subject to the laser beam and released the contents of bacteria cells on media [16,18].

Another reason for such a decrease is the thermal effect on the bacteria, which is produced by laser beam. This heating makes vacuoles inside the cell that leads to the killing of bacteria. Furthermore, laser is able to produce some changes at both photochemical and photobiological levels in bacterial cells, thus functions to reduce the number of bacterial colonies [10,17].

Fig. 1a shows that the control sample contains uncountable number of bacteria colonies, which means that it is more than 500 colonies. When the sample was exposing to laser, the number of colonies started to be counted and then get reduced as a function of exposure time. However, the colonies were not affected as happens to the sample №A2 due to the difference in both exposure time and laser power, which is reduced dramatically from the control, Fig. 2a to exposure of 30 minutes as in Fig. 2d. The results show that using He-Ne with a wavelength of 632.8 nm at 3 mW for 30 minutes seems more effective to reduce the amount of bacteria to the less than 2% of the initial count. This facilitates and speeds up healing from the *S. aureus* bacteria related diseases. Nevertheless, it is well-known that this bacterium is involved in so many skin infections, thus it can be easily treated using laser as skin can be exposed directly to the laser and doesn't require sophisticated precautions.

The bactericidal effect of He-Ne laser was reported in some studies to occur due to the production of reactive oxygen species (ROS) in the cells of the bacteria, which leads to cell death. Furthermore, the bactericidal effect of He-Ne lasers appears to be dependent on the intensity and duration of the laser treatment, with higher intensities and longer durations generally resulting in greater bactericidal activity. Some studies have suggested that the bactericidal effect of He-Ne lasers may be enhanced by the use of photosensitizers, which can increase the production of ROS in bacteria when exposed to light [21].

The current results allowed concluding that the laser at a wavelength of 632.8 nm has a bactericidal effect on *S. aureus*, which is involved in several dermatology problems. Using He-Ne with a wavelength of 632.8 nm at 3 mW for 30 minutes seems to be effective to reduce the amount of bacteria to less than 2% of the initial count.

Conclusion

The bactericidal effect of 632.8 nm low level laser on *S. aureus* was examined through exposing several bacterial colonies to He-Ne laser at different parameters. The results showed that more than 98% of the bacteria are killed when a wavelength of 632.8 nm at 3 mW for 30 minutes is used. This can be attributed to the production of reactive oxygen species in the cells of the bacteria which is reported in the literature to be associated with the laser intensity, power and exposure time. The research on the bactericidal effect of 632.8 nm low level laser at certain power and exposure time could be elaborated to find its way to clinical application since *S. aureus* is reported to be involved in many dermatological diseases.

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REFERENCES

1. Guffey JS, Wilborn J. In Vitro Bactericidal Effects of 405-nm and 470-nm Blue Light. *Photomed Laser Surg*, 2006, Vol. 24(6), pp.684-8
2. Semyonov D.Yu., Vasil'ev Yu.L., Dydykin S.S., Stranadko E.F., Shubin V.K., Bogomazov Yu.K., Morokhotov V.A., Shcherbyuk A.N., Morozov S.V., Zakharov Yu.I. Antimicrobial and antimycotic photodynamic therapy (review of literature). *Biomedical Photonics*, 2021, Vol. 10(1), pp. 25-31. doi: 10.24931/2413–9432–2021–10–1–25–31
3. Zhidomorov N.Yu., Nazarenko O.A., Demidov V.I., Kustov A.V., Kukushkina N.V., Koifman O.I., Gagua A.K., Tomilova I.K., Berezin D.B. Study of acute toxicity of monocationic chlorin e6 derivative, a perspective photosensitizer for antimicrobial and antitumor photodynamic therapy. *Biomedical Photonics*, 2022, Vol. 11(2), pp. 23-32. doi: 10.24931/2413–9432–2022–11-2-23-32
4. Shinkarev S.A., Boldyrev S.V., Zagadaev A.P., Podolsky V.N., Borisov V.A., Kostyushina Y.I. Antimicrobial photodynamic therapy in voice rehabilitation of patients after laryngectomy. *Biomedical Photonics*, 2021, Vol. 10(1), pp. 11-16. doi: 10.24931/2413–9432–2021–10–1–11–16
5. Nussbaum EL, Lilge L, Mazzulli T. Effects of 630-, 660-, 810-, and 905-nm Laser Irradiation Delivering Radiant Exposure of 1-50 J/cm2 on Three Species of Bacteria in Vitro. *J Clin laser Med \& Surg*, 2002, Vol. 20(6), pp. 325-33
6. Glala N. Biological effect of the laser-fuandmantal and basic study on bacteria, 2004

ЛИТЕРАТУРА

1. Guffey JS, Wilborn J. In Vitro Bactericidal Effects of 405-nm and 470-nm Blue Light. *Photomed Laser Surg*, 2006, Vol. 24(6), pp.684-8
2. Semyonov D.Yu., Vasil'ev Yu.L., Dydykin S.S., Stranadko E.F., Shubin V.K., Bogomazov Yu.K., Morokhotov V.A., Shcherbyuk A.N., Morozov S.V., Zakharov Yu.I. Antimicrobial and antimycotic photodynamic therapy (review of literature). *Biomedical Photonics*, 2021, Vol. 10(1), pp. 25-31. doi: 10.24931/2413–9432–2021–10–1–25–31
3. Zhidomorov N.Yu., Nazarenko O.A., Demidov V.I., Kustov A.V., Kukushkina N.V., Koifman O.I., Gagua A.K., Tomilova I.K., Berezin D.B. Study of acute toxicity of monocationic chlorin e6 derivative, a perspective photosensitizer for antimicrobial and antitumor photodynamic therapy. *Biomedical Photonics*, 2022, Vol. 11(2), pp. 23-32. doi: 10.24931/2413–9432–2022–11-2-23-32
4. Shinkarev S.A., Boldyrev S.V., Zagadaev A.P., Podolsky V.N., Borisov V.A., Kostyushina Y.I. Antimicrobial photodynamic therapy in voice rehabilitation of patients after laryngectomy. *Biomedical Photonics*, 2021, Vol. 10(1), pp. 11-16. doi: 10.24931/2413–9432–2021–10–1–11–16
5. Nussbaum EL, Lilge L, Mazzulli T. Effects of 630-, 660-, 810-, and 905-nm Laser Irradiation Delivering Radiant Exposure of 1-50 J/cm2 on Three Species of Bacteria in Vitro. *J Clin laser Med \& Surg*, 2002, Vol. 20(6), pp. 325-33
6. Glala N. Biological effect of the laser-fuandmantal and basic study on bacteria, 2004

7. Song HJ, Seo HJ, Kim D. Effectiveness of high-intensity laser therapy in the management of patients with knee osteoarthritis: A systematic review and meta-analysis of randomized controlled trials. *Journal of Back and Musculoskeletal Rehabilitation. IOS Press BV*, 2020, Vol. 33, pp. 875-84
8. Niemz MH. Laser-Tissue Interactions: Fundamentals and Applications. *Springer Berlin Heidelberg*, 2013 (Biological and Medical Physics, Biomedical Engineering)
9. Eissa M, Salih WHM. The influence of low-intensity He-Ne laser on the wound healing in diabetic rats. *Lasers Med Sci*, 2017, Vol.32(6), pp. 1261-1267
10. Lubart R, Lipovski A, Nitzan Y, Friedmann H. A possible mechanism for the bactericidal effect of visible light. *Laser Ther*, 2011, Vol.20(1), pp. 17-22
11. Ribeiro M, Da Silva D, De Araújo C, De Oliveira S, Pelegrini C, Zorn TM, et al. Effects of Low-Intensity Polarized Visible Laser Radiation on Skin Burns A Light Microscopy Study. *J Clin laser Med surgry*, 2004, Vol.22(1), pp. 59-66
12. Folwaczny M, Mehl A, Aggstaller H, Hickel R. Anti microbial effectes of 2.94 microm Er:YAG Laser radiation on root surface: invitro study. *J Clin Periodontol*, 2002, Vol.29(1), pp. 73-78
13. Wilson M, Yianni C, Wilson, Yianni. Killing of methicillin-resistant Staphylococcus aureus by low-power. *J Med Microbiol*, 1995, Vol.42(1), pp. 62-66
14. Avram M, Rogers N. The use of low-level light for hair growth Part I. *J Cosmet laser Ther*, 2009, Vol.11(2), pp. 110-7
15. Avaci P, Gupta G, Clark J, Wikonkal J, Wikonkal N, Hamblin M // Low-level laser (light) therapy (LLLT) for treatment of hair loss. *Lasers Surg Med*, 2014, Vol.46(2), pp.144-151
16. Karu T, Tiphlova O, Esenaliev R, Letokhov V. Two different mechanisms of low-intensity laser photobiological effects on Escherichia coli. *J Photochem Photobiol B Biol*, 1994. – Vol.24(1). – P.55-61
17. Nandakumar K, Obika H, Shinozaki T, Ooie T, Utsumi A, Yano T. Laser Impact on Bacterial ATP : Insights into the Mechanism of Laser-Bacteria Interactions. *Biofouling*, 2003, Vol.19(2), pp.109-114
18. Fadhali MM, Saeed FA, Hashim NM, Toto S, Ali J. Investigation of Laser Induced Inhibition and Simulation in Biological Samples. *Opt Photonics J*, 2011, Vol. 01(03), pp.101-105.
19. Fekrazad R, Nejat AH, Kalhori KAM. Antimicrobial Photodynamic Therapy With Nanoparticles Versus Conventional Photosensitizer in Oral Diseases. In: Nanostructures for Antimicrobial Therapy: Nanostructures in Therapeutic Medicine Series. *Elsevier*, 2017, pp. 237-259
20. Atluri R, Korir D, Choi T-Y, Simmons DP. Empirical Studies on Effect of Low-Level Laser Treatment on Glioblastoma Multiforme in Combination with Ag-PMMA-PAA Nanoparticles: Paired Red Region Optical-Property Treatment Platform. *Appl Nano*, 2022, Vol.3(2), pp.112-125
21. Zhao X, Drlica K. Reactive oxygen species and the bacterial response to lethal stress. Vol. 21, Current Opinion in Microbiology. *Elsevier Ltd*, 2014, pp. 1-6

7. Song HJ, Seo HJ, Kim D. Effectiveness of high-intensity laser therapy in the management of patients with knee osteoarthritis: A systematic review and meta-analysis of randomized controlled trials. *Journal of Back and Musculoskeletal Rehabilitation. IOS Press BV*, 2020, Vol. 33, pp. 875-84
8. Niemz MH. Laser-Tissue Interactions: Fundamentals and Applications. *Springer Berlin Heidelberg*, 2013 (Biological and Medical Physics, Biomedical Engineering)
9. Eissa M, Salih WHM. The influence of low-intensity He-Ne laser on the wound healing in diabetic rats. *Lasers Med Sci*, 2017, Vol.32(6), pp. 1261-1267
10. Lubart R, Lipovski A, Nitzan Y, Friedmann H. A possible mechanism for the bactericidal effect of visible light. *Laser Ther*, 2011, Vol.20(1), pp. 17-22
11. Ribeiro M, Da Silva D, De Araújo C, De Oliveira S, Pelegrini C, Zorn TM, et al. Effects of Low-Intensity Polarized Visible Laser Radiation on Skin Burns A Light Microscopy Study. *J Clin laser Med surgry*, 2004, Vol.22(1), pp. 59-66
12. Folwaczny M, Mehl A, Aggstaller H, Hickel R. Anti microbial effectes of 2.94 microm Er:YAG Laser radiation on root surface: invitro study. *J Clin Periodontol*, 2002, Vol.29(1), pp. 73-78
13. Wilson M, Yianni C, Wilson, Yianni. Killing of methicillin-resistant Staphylococcus aureus by low-power. *J Med Microbiol*, 1995, Vol.42(1), pp. 62-66
14. Avram M, Rogers N. The use of low-level light for hair growth Part I. *J Cosmet laser Ther*, 2009, Vol.11(2), pp. 110-7
15. Avaci P, Gupta G, Clark J, Wikonkal J, Wikonkal N, Hamblin M // Low-level laser (light) therapy (LLLT) for treatment of hair loss. *Lasers Surg Med*, 2014, Vol.46(2), pp.144-151
16. Karu T, Tiphlova O, Esenaliev R, Letokhov V. Two different mechanisms of low-intensity laser photobiological effects on Escherichia coli. *J Photochem Photobiol B Biol*, 1994. – Vol.24(1). – P.55-61
17. Nandakumar K, Obika H, Shinozaki T, Ooie T, Utsumi A, Yano T. Laser Impact on Bacterial ATP : Insights into the Mechanism of Laser-Bacteria Interactions. *Biofouling*, 2003, Vol.19(2), pp.109-114
18. Fadhali MM, Saeed FA, Hashim NM, Toto S, Ali J. Investigation of Laser Induced Inhibition and Simulation in Biological Samples. *Opt Photonics J*, 2011, Vol. 01(03), pp.101-105.
19. Fekrazad R, Nejat AH, Kalhori KAM. Antimicrobial Photodynamic Therapy With Nanoparticles Versus Conventional Photosensitizer in Oral Diseases. In: Nanostructures for Antimicrobial Therapy: Nanostructures in Therapeutic Medicine Series. *Elsevier*, 2017, pp. 237-259
20. Atluri R, Korir D, Choi T-Y, Simmons DP. Empirical Studies on Effect of Low-Level Laser Treatment on Glioblastoma Multiforme in Combination with Ag-PMMA-PAA Nanoparticles: Paired Red Region Optical-Property Treatment Platform. *Appl Nano*, 2022, Vol.3(2), pp.112-125
21. Zhao X, Drlica K. Reactive oxygen species and the bacterial response to lethal stress. Vol. 21, Current Opinion in Microbiology. *Elsevier Ltd*, 2014, pp. 1-6

REVIEWS OF LITERATURE

PHOTODYNAMIC THERAPY OF PSORIASIS

Filonenko E.V.[1], Ivanova-Radkevich V.I.[1,2]
[1]P.A. Herzen Moscow Oncology Research Center – branch of FSBI NMRRC of the Ministry of Health of the Russian Federation, Moscow, Russia
[2]Peoples' Friendship University of Russia (RUDN University), Moscow, Russia

Abstract

Photodynamic therapy (PDT) in the treatment of psoriasis remains the subject of much debate. There is no consensus in the scientific community about effective and safe PDT regimens for psoriasis. Described in the published materials doses and concentrations of photosensitizers for psoriasis, as well as light doses, differ by dozens of times. The purpose of this review is to analyze the efficacy and safety profile of various PDT regimens for psoriasis. Some studies demonstrate 100% effectiveness of the method in certain modes (complete or partial clearance of psoriasis foci after PDT). In particular, such efficiency was obtained with the application of 20% 5-ALA (light dose 15 J/cm^2) and 0.1% methylene blue (light dose 15 J/cm^2). The main factor limiting the use of PDT in psoriasis, and in some cases even being the reason for treatment interruption, is severe pain during the irradiation procedure. This requires careful development of PDT regimens in patients with psoriasis.

Key words: photodynamic therapy, psoriasis, 5-aminolevulinic acid, 5-aminolevulinic acid methyl ester.

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Contacts: Filonenko E.V., e-mail: derkul23@yandex.ru

ФОТОДИНАМИЧЕСКАЯ ТЕРАПИЯ БОЛЬНЫХ ПСОРИАЗОМ

Е.В. Филоненко[1], В.И. Иванова-Радкевич[2]
[1]«Московский научно-исследовательский онкологический институт им. П.А. Герцена – филиал ФГБУ «Национальный медицинский исследовательский центр радиологии» Министерства здравоохранения Российской Федерации, Москва, Россия
[2]Российский Университет дружбы народов, Москва, Россия

Резюме

Использование фотодинамической терапии (ФДТ) в лечении псориаза остается предметом многочисленных дискуссий. В научном сообществе нет единого мнения об эффективных и безопасных режимах ФДТ при псориазе. Описанные в литературе применяемые для лечения псориаза дозы и концентрации фотосенсибилизаторов, а также световые дозы различаются в десятки раз. Целью настоящего обзора является анализ эффективности и профиля безопасности различных схем применения ФДТ при псориазе. Ряд исследований демонстрирует 100%-ную эффективность метода в определенных режимах (полное или частичное очищение очагов псориаза после проведения ФДТ). В частности, такая эффективность была получена при применении аппликации 20%-ой 5-АЛК (световая доза 15 Дж/см^2) и 0,1%-го метиленового синего (световая доза 15 Дж/см^2). Основным фактором, ограничивающим применение ФДТ при псориазе и в отдельных случаях даже являющимся причиной прерывания лечения, является сильная болезненность во время процедуры облучения. Это требует тщательной отработки режимов ФДТ у пациентов с псориазом.

Ключевые слова: фотодинамическая терапия, псориаз, 5-аминолевулиновая кислота, метиловый эфир 5-аминолевулиновой кислоты.

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Контакты: Филоненко Е.В., e-mail: derkul23@yandex.ru

Introduction

Psoriasis is a systemic immune-associated disease of a multifactorial nature with a dominant role in the development of genetic factors, characterized by accelerated proliferation of epidermocytes and a violation of their differentiation, immune reactions in the dermis and synovial membranes, an imbalance between pro-inflammatory and anti-inflammatory cytokines, chemokines; frequent pathological changes in the musculoskeletal system [1].

Epidemiology

Psoriasis is one of the most common skin diseases. The disease is equally common in both men and women [2]. Data on the incidence of psoriasis vary significantly in different regions, from 0.14% in East Asia to 5.32% in Central Europe [3].

In general, the incidence is higher in the countries of Eastern Europe and the countries of the Scandinavian Peninsula [4]. A low incidence rate is observed among people in Asia and Africa, in African Americans, Indians, and Japanese [5].

In the Russian Federation in 2020, the prevalence of psoriasis among the entire population was 227.2 per 100 thousand population, and the incidence was 52.5 per 100 thousand population [6]. According to official state statistics in the Russian Federation, the prevalence of psoriasis in 2021 was 243.7 diseases per 100 thousand population, and the incidence was 59.3 per 100 thousand population [1].

Etiology and pathogenesis

The development of psoriasis is primarily associated with genetic predisposition, autoimmune disorders, and environmental factors, including infections and stress [7,8,9]. The pathogenesis of psoriasis is a multifactorial process. One of the factors determining the development of psoriasis is an increase in the expression of pro-inflammatory cytokines. For example, interleukin 17 and interleukin 23 stimulate keratinocyte proliferation and increase the secretion of TNF-α and chemokines that enhance dendritic cell activation, leading to inflammation [9,10,11,12].

Clinical manifestation

Psoriasis is characterized by diverse clinical manifestations from single abundantly scaly papules or pinkish-red plaques to erythroderma, psoriatic arthritis, and generalized or limited pustular psoriasis. Rashes can be located on any part of the skin, but most often, they appear on the extensor surface of the limbs, scalp, and torso. Psoriatic papules vary in size, intensity of the inflammatory reaction, and infiltration that can be very significant and be accompanied by papillomatous and warty growths. In addition to the skin and joints, psoriasis also affects the nail plates [13].

There are three stages in the development of psoriatic rashes [14]: 1) a period of progression or "bloom", when the elements of the rash continue to increase in size, and this usually coincides with the appearance of new rashes and a hyperemic border along their periphery; 2) a stationary period, when the peripheral growth of rashes has stopped, which usually coincides with the cessation of the appearance of fresh rashes; 3) a period of regression, or reverse the development of rashes. It should be noted that the allocation of three stages in the development of psoriatic rashes is only a scheme and there are often deviations from it.

Therapy for psoriasis

Depending on the type of psoriasis, its localization, degree, and severity, various therapy regimens are used for treatment, including topical preparations (based on salicylic acid, vitamin A, tar, etc.), local and systemic use of corticosteroids, calcipotriene, oral systemic preparations (e.g. acitretin, cyclosporine, methotrexate), biologics (etanercept, infliximab, alefacept, efalizumab, and ustekinumab), as well as ultraviolet B (UVB) phototherapy and PUVA therapy [13,15,16].

Studies show high efficacy in the treatment of psoriasis of narrow-band ultraviolet B (NB-UVB, 311 nm) and even excimer laser (308 nm) used as a monochromatic UVB source [17]. These methods are currently used as first-line therapy for stable plaque psoriasis. The first-line therapy for the treatment of refractory psoriatic plaques is PUVA therapy [18].

PDT

Photodynamic therapy (PDT) appears to be an attractive treatment option for psoriasis primarily due to its general and cost-effectiveness [15]. The effectiveness of PDT against various tumor and precancerous skin diseases (including basal cell skin cancer [19], extramammary Paget's cancer [20], and mycosis fungoides [21]) has been proven by numerous studies. At the same time, the use of PDT in the treatment of psoriasis remains the subject of numerous discussions. There is no consensus in the scientific community about effective and safe PDT regimens for psoriasis. In different studies, compounds of different chemical groups are used as photosensitizers in doses and concentrations that differ dozens of times. For example, the effectiveness of the topical application of 5-ALA preparations at concentrations from 0.1% to 20% is described. The range of light dose used is also very wide (from 2 to 37 J/cm^2). The total number of PDT courses and the duration of the intervals between courses also differ.

The purpose of this review is to analyze the efficacy and safety profile of various PDT regimens. A comparison of recent advances in this regard seems to be useful for the further development of the PDT method for the treatment of psoriasis.

Analysis of the published data did not reveal any studies showing a 100% recovery in patients with psoriasis treated with PDT. However, several studies demonstrate 100% efficiency of the method in certain modes (complete or partial clearance of psoriasis foci after PDT), that is, all psoriasis foci respond (to a greater or lesser extent) to PDT in certain modes. A very important argument in favor of the use of PDT in psoriasis is the fact that several studies have shown that PDT blocks the uncontrolled production of inflammatory cytokines that lead to T-lymphocyte apoptosis and inflammation during the development of psoriasis [22,23]. Even in conditions of incomplete purification of psoriasis foci, a decrease in the intensity of the inflammatory process in them certainly alleviates the condition of patients.

The analysis of the scientific data made it possible to identify 14 studies on the efficacy and safety of PDT with various photosensitizers in patients with psoriasis. Twelve studies assessed the effectiveness of PDT against skin lesions of psoriasis, and two studies assessed the effectiveness of PDT against nail psoriasis. The analysis did not include studies in which PDT was performed in combination with other therapies since the results of such studies do not allow to assess the contribution to the effectiveness of PDT. Comparison of the effectiveness of individual PDT regimens was difficult due to the different assessment methods used in the studies. In part of the studies, the condition of patients was characterized using various indices (NAPSI, SEI, and others), and in the other part, the effectiveness was assessed as complete or partial clearance of lesions.

In most of PDT studies for skin manifestations of psoriasis (9/12), 5-ALA was used as a photosensitizer. In 8 cases it was used in the form of an application, and in 1 case it was given in the form of an oral solution. The concentration of the dosage form of 5-ALA for application varied significantly, from 0.1% (1 study) to 20% (4 studies). The effectiveness of PDT with the application of 5-ALA at a concentration of 20% was higher than for lower doses: up to 100% complete or partial clearance of psoriasis foci using 20% 5-ALA [24] versus only partial improvement in 37.5% of patients after application of 5-ALA at a concentration of 0.1% [25]. At the same time, in a study by Radakovic-Fijan et al. [26] when using 5-ALA at a concentration of 1%, the overall efficiency (completely or partially cleared foci) was 97%, which is very close to the results of PDT for a concentration of 5-ALA 20% (efficiency 100%) [24].

Studies were also analyzed that evaluated the effectiveness of methylene blue (0.1%) [27], hypericin (0.05-0.25%) [28], and 5-ALA methyl ester (ME-ALA) (16%) [29] (one study of each photosensitizer). All of the listed photosensitizers showed high efficiency. Thus, after PDT with methylene blue in 68% of patients with psoriasis, a decrease in the severity of psoriasis by 75% or more was obtained [27].

The only reported adverse events in all studies were pain, itching, and burning during the irradiation session, and in some patients for some time after irradiation. For several patients the study has been discontinued due to severe pain (3/12 patients in the study by Schleyer et al. [25] and 8/29 patients in the study by Radakovic-Fijan et al. [26]). Even though many authors associate the development of pain with the use of high light doses and a high concentration of a photosensitizer, there is no definite certainty in this connection. For example, in a study by Calzavara-Pinton et al. [29], in which the highest light doses of all considered studies (37 J/cm^2) and the highest concentration of 5-ALA (20%) were applied, only 4 out of 17 patients experienced severe pain, and not a single patient came out of studies due to severe pain. It seems more likely that pain sensations may be related to the radiation power density. In both studies, in which some patients had to discontinue treatment, the power density was 60 mW/cm^2. Unfortunately, the radiation power density for PDT was indicated not in all the studies, thus it is not possible to reliably assess the relationship between this indicator and the intensity of pain sensations.

When performing PDT of nails affected by psoriasis, researchers used higher concentrations of photosensitizers or higher light doses. In a study by Shaheen et al. [30] nails were treated with 2% methylene blue solution, in a study by Tehranchinia et al. [31] the light dose was 120 J/cm^2 after application of 5-ALA at a standard concentration of 20%. It should be noted that during irradiation of nails affected by psoriasis, patients did not notice severe pain as it was in PDT of skin foci, even when using a light dose significantly higher than in all other studies (120 J/cm^2). The effectiveness of PDT in both studies was assessed by the NAPSI index, which decreased after PDT by 1.6-2.8 times.

New photosensitizers for PDT in psoriasis

The results of several experimental studies on the evaluation of the efficacy and safety of new photosensitizers in the treatment of psoriasis have been published. Carrenho L.Z.B. et al. [32] report an immunosuppressive effect of a form of porphyrin (5,10-diphenyl-15,20-di(N-methylpyridinium-4-yl)porphyrin) in a mouse model of psoriasis. According to this study, PDT with the specified photosensitizer led to a decrease in the level of pro-inflammatory cytokines, neutrophil infiltration, and proliferation of keratinocytes [32]. In a study by Liu H.Q. et al. [33] the effectiveness of the photosensitizer α-(8-quinolinoxy)phthalocyanine zinc against psoriasis was evaluated. The authors report a decrease in HaCaT cell proliferation and IL-17 mRNA expression after PDT with the indicated photosensitizer. Another group of photosensitizers promising for the treatment of psoriasis are complexes based on NNO-vanadium (IV) tridentate. Lin R.K. et al. [34] demonstrated the anti-inflammatory effects of PDT with these photosensitizers in a mouse model of psoriasis-like skin disease. After PDT, the expression of cytokines IL-17 and IL-22 decreased, which indicates the possibility of alleviating the symptoms of psoriasis.

Таблица
Резюме эффективности фотодинамической терапии у больных псориазом
Table
Summary of the effectiveness of photodynamic therapy in patients with psoriasis

Авторы Authors	Число пациентов* / количество очагов / No. of patients/ No. of lesions	Фотосенсибилизатор Photosensitizer	Режим облучения Light wavelength	Световая доза Light dose	Количество курсов ФДТ Number of PDT courses	Эффективность ФДТ PDT efficiency	Нежелательные реакции Adverse reactions
Boehncke et al., 1994 [35]	3/не указано 3/not specified	10% 5-АЛК, аппликация 5 ч 10% 5-ALA, application 5 h	600-700 нм 600-700 nm	25 Дж/см^2 25 J/cm^2	3 раза в нед 3 times a week	Эффективность ФДТ сопоставима с применением дитранола The effectiveness of PDT is comparable to the use of dithranol	Жжение во время облучения Burning during radiation
Collins et al., 1997 [36]	22/80	20% 5-АЛК, аппликация 4 ч 20% 5-ALA, application 4 h	450-600 нм 450-600 nm	2-16 Дж/см^2 2-16 J/cm^2	12 (3 раза в нед) 12 (3 times a week)	Эффективность (полный или частичный эффект) 25%. Из 80 очагов: 14 – полное очищение; 6 – снижение индекса SEI (ат англ. scale, erythema, and induration – шелушение, эритрема, отвердение) на 30-50%; 60 – незначительное улучшение или отсутствие ответа Efficiency (full or partial effect) – 25%. Of the 80 foci: 14 – complete cleansing; 6 – decrease in the SEI index (at English scale, erythema, and induration – peeling, erythrema, hardening) by 30-50%; 60 – slight improvement or no response	Жжение, покалывание во время и после облучения Burning, tingling during and after irradiation
Robinson et al., 1999 [37]	10/19	20% 5-АЛК, аппликация 4 ч 20% 5-ALA, application 4 h	Широкополосное видимое излучение Broad-band visible radiation	8 Дж/см^2 8 J/cm^2	12 (3 раза в нед) 12 (3 times a week)	Эффективность (полный или частичный эффект) – 74% Из 19 очагов: 4 – полное очищение; 10 – частичный эффект; 5 – отсутствие ответа Efficiency (full or partial effect) – 74% Of the 19 foci: 4 – complete cleansing; 10 – partial effect; 5 – no response	Боль и дискомфорт (80% пациентов во время лечения и 50% между процедурами) Pain and discomfort (80% of patients during treatment and 50% between treatments)
Bissonnette et al., 2002 [38]	12/не указано 12/not specified	Раствор 5-АЛК внутрь 5-15 мг/кг 5-ALA solution orally, 5-15 mg/kg	417 нм 417 nm	1-20 Дж/см^2 Dose, 1-20 J/cm^2	1 раз в нед 1 time per week	Только доза 15 мг/кг показала улучшение состояния пациентов Only the 15 mg/kg dose showed improvement in patients	Легкое жжение при воздействии света Mild burning on exposure to light

Radakovic-Fijan, 2005 [26]	21/63 21/63	1% 5-АЛК, аппликация 4-6 ч 1% 5-ALA, application 4-6 h	600-740 нм 600-740 nm	5; 10; 20 Дж/см² 5; 10; 20 J/cm²	2 раза в нед, 6 нед 2 times a week, 6 weeks	Эффективность (полный или частичный эффект) – 97% Из 63 очагов: 8 – полное очищение; 53 – частичный эффект; 2 – отсутствие ответа Efficiency (full or partial effect) – 97% Of the 63 foci: 8 – complete cleansing; 53 – partial effect;w2 – no response	Боль, покалывание, жжение во время облучения и несколько часов после у всех пациентов (8 из исходно включенных в исследование 29 пациентов прекратили лечение в связи с сильными болевыми ощущениями) Pain, tingling, burning sensation during and several hours after exposure in all patients (8 out of 29 patients initially included in the study discontinued treatment due to severe pain)
Fransson et. al., 2005 [39]	8/8 8/8	20% 5-АЛК, аппликация 4-5 ч 20% 5-ALA, application 4-5 h	630 нм 630 nm	10-30 Дж/см² 10-30 J/cm²	1 раз в нед, 2-5 нед 1 time per week, 2-5 weeks	Индекс SEI значительно снизился у всех пациентов, с медианы 7 (диапазон 5-9) до 1,5 (диапазон 0-3) The SEI index decreased significantly in all patients, from a median of 7 (range 5-9) to 1.5 (range 0-3)	При дозе света 30 Дж/см² многие пациенты испытывали болезненные ощущения, поэтому световая доза была снижена At a light dose of 30 J/cm², many patients experienced pain, the light dose was reduced
Schleyer et al., 2006 [25]	9/27 9/27	0,1%, 1% и 5% 5-АЛК, аппликация 0.1%, 1% and 5% of 5-ALA, application	600-740 нм 600-740 nm	20 Дж/см² 20 J/cm²	2 раза в нед, 6 нед 2 times a week, 6 weeks	Полного очищение не зарегистрировано. Частичное улучшение: 0,1% 5-АЛК – 37,5%; 1% 5-АЛК – 45,6%; 5% 5-АЛК – 51,2% Complete clearance has not been recorded. Partial improvement: 0.1% 5-ALA – 37.5%; 1% 5-ALA – 45.6%; 5% 5-ALA – 51.2%	Сильные болевые ощущения у всех пациентов (3 из исходно включенных в исследование 12 пациентов прекратили лечение в связи с сильными болевыми ощущениями) Severe pain in all patients (3 out of 12 patients initially included in the study discontinued treatment due to severe pain)
Smits et al., 2006 [40]	8/8 8/8	10% 5-АЛК, аппликация 4 ч 10% 5-ALA, application 4 h	600-750 нм 600-750 nm	2-8 Дж/см² 2-8 J/cm²	1 раз в нед, 4 нед 1 time per week, 4 weeks	По 12 бальной шкале исходный индекс степени поражения псориаза в среднем составил 6,6. Через 6 нед в очагах без воздействия фотосенсибилизатора индекс составил 6,2, а для очагов, обработанных ФДТ – 4,2 On a 12-point scale, the initial index of the degree of psoriasis lesions averaged 6.6. After 6 weeks, in the lesions without exposure to the photosensitizer, the index was 6.2, and for the lesions treated with PDT – 4.2	Некоторое жжение и покалывание во время облучения, в целом лечение хорошо переносилось всеми пациентами, и никаких дополнительных анальгетиков не требовалось Some patients experienced some burning and stinging during the irradiation, generally the treatment was well tolerated by all patients and no additional analgesics were needed

Kim et al., 2007 [24]	3/не указано 3/not specified	20% 5-АЛК, аппликация 4 ч 20% 5-ALA, application 4 h	630 нм 630 nm	15 Дж/см² 15 J/cm²	1 раз в нед, 7, 10 и 23 нед Once a week, 7, 10 and 23 weeks	Эффективность (полный или частичный эффект) 100%. После лечения во всех случаях наблюдалось незначительное или заметное улучшение Efficiency (full or partial effect) 100%. After treatment, in all cases there was a slight or noticeable improvement	Не сообщалось Not reported
Salah et al., 2009 [27]	16/16 16/16	0,1% метиленовый синий аппликация 0.1% of methylene blue, application	670 нм 670 nm	5 Дж/см² 5 J/cm²	Нет данных No data available	Эффективность (полный или частичный эффект) 100% Efficiency 100% (full or partial effect)	Не сообщалось Not reported
Rook et al., 2009 [28]	11/не указано 11/not specified	0,05%, 0,1% и 0,25% гиперицин, аппликация, 24 ч 0.05%, 0.1% and 0.25% of hypericin, application, 24 h	590-650 нм 590-650 nm	8-20 Дж/см² 8-20 J/cm²	2 раза в нед, 3 нед 2 times a week, 3 weeks	Наблюдалось улучшение кожных поражений An improvement in skin lesions	Легкое жжение и зуд во время лечения Mild burning and itching during treatment
Calzavara-Pinton et al., 2013 [29]	17/не указано 17/not specified	МЭ-АЛК 16% аппликация 3-4 ч MAL 16% application for 3-4 h	635 нм 635 nm	37 Дж/см² 37 J/cm²	В среднем 3,6 (интервал между курсами 9,9±5,6 дней) On average 3.6 courses (interval between courses 9.9±5.6 days)	Из 17 пациентов: у 2 – ухудшение состояния; у 3 – незначительное клиническое улучшение; у 12 – существенное клиническое улучшение. У 5 (28%) косметический эффект оценен, как отличный Of 17 patients: in 2 – deterioration; in 3 – slight clinical improvement; 12 had significant clinical improvement. In 5 (28%), the cosmetic effect was rated as excellent	Боль и жжение в период облучения у 13 (76%) пациентов, в том числе у 4 – слабые, у 4 – умеренные, у 4 – сильные Pain and burning sensation during irradiation in 13 (76%) patients, including 4 mild, 4 moderate, 4 severe
Shaheen et al., 2023 [30]	29/ногти правой руки 29/nails of the right hand	2% метиленовый синий, аппликация 2 ч 2% of methylene blue, application for 2 h	585 нм 585 nm	15 Дж/см² 15 J/cm²	1 раз в 2 нед, 6 мес 1 time in 2 weeks, 6 months	Показатели индекса NAPSI для матрицы ногтя снизились в среднем от 7 до 4,5 The NAPSI index for the nail matrix decreased from on average from 7 to 4.5	Небольшие болевые ощущения во время сеанса облучения Slight pain during the radiation session
Tehran-chinia et al., 2020 [31]	8/35 ногтей 8/35 nails	20% АЛК, аппликация 3 ч 20% of 5-ALA, application 3 h	630 нм 630 nm	120 Дж/см² 120 J/cm²	1 раз в 3 нед, 5 курсов 1 time in 3 weeks, 5 courses	Показатели NAPSI значительно снизились с 5,97±1,29 в начале исследования до 4,29±1,44 на 15-й неделе и 2,11±1,27 в конце 24-й нед наблюдения после завершения ФДТ NAPSI scores significantly decreased from 5.97±1.29 at baseline to 4.29±1.44 at week 15 and 2.11±1.27 at the end of week 24 after completion of PDT	Не сообщалось о сильной боли и дискомфорте во время облучения No severe pain or discomfort was reported during irradiation

*указано число пациентов, завершивших исследование с оцененным эффектом
*the number of patients who completed the study with an estimated effect is indicated

The analyzed data leave no doubt that PDT is an effective and promising treatment for psoriasis. The issue under discussion is the choice of the optimal photosensitizer, its dose (concentration for application), light dose, and irradiation regimen.

Since there is evidence that protoporphyrin IX (PpIX) accumulates with very high selectivity in psoriasis lesions [40], it can be assumed that lower concentrations of 5-ALA than those used for dermato-oncological indications may be sufficient to provide a beneficial clinical effect in psoriasis. This is supported by data from studies in which 5-ALA was used at a low concentration (1%) [26] but with an efficiency close to studies of 20% 5-ALA [27]. In addition, as some authors [40] believe, the main purpose of PDT in psoriasis is probably not a cytotoxic effect, which requires higher light doses, but rather an immunomodulatory effect, which is believed to require repeated exposure to lower photodynamic doses over a longer period.

REFERENCES

1. Clinical recommendations of the Russian Federation 2023 «Psoriasis».
2. Kubanova A.A., Kisina V.I., Blatun L.A. Rational pharmacotherapy of skin diseases and sexually transmitted infections. *Guidelines for practicing physicians*, 2005, p. 882.
3. Lowes M.A., Suárez-Fariñas M., Krueger J.G. Immunology of psoriasis. *Annu. Rev. Immunol,* 2014, vol. 32, pp. 227-255 doi: 10.1146/annurev-immunol-032713-120225
4. Usatine R.P., Smith M.A., Maye E.J. Atlas of a practicing physician. *Dermatology*, 2012, p. 536.
5. Kay Sh.K., Stratigos D.A., Lio P.A., Johnson R.A. Pediatric dermatology. *Color Atlas and handbook / Kay Shu-Mei Kane et al.; translated from English. edited by O.L. Ivanov, A.N. Lvova. – M.: Panfilov Publishing House; BINOM. Laboratory of knowledge*, 2011, p. 496.
6. Kubanov A.A., Bogdanova E.V. Dermatovenereology of Russian Federation in 2020: Working Under a Pandemic. *Vestnik Dermatologii i Venerologii*, 2021, vol. 97(4), pp. 8-32. doi: https://doi.org/10.25208/vdv1261
7. Zhang P., Wu M.X. A clinical review of phototherapy for psoriasis. *Lasers Med. Sci,* 2018, vol. 33, pp. 173-180. doi: 10.1007/s10103-017-2360-1
8. Campbell J. Safe and effective use of phototherapy and photochemotherapy in the treatment of psoriasis. *Br. J. Nurs*, 2020, vol. 29, pp. 547-552. doi: 10.12968/bjon.2020.29.10.547
9. Olisova O.Yu., Garanyan L.G. Epidemiology, etiopathogenesis, comorbidity in psoriasis – new facts. *Russian Journal of Skin and Venereal Diseases (Rossiyskii Zhurnal Kozhnykh i Venericheskikh Boleznei)*, 2017, vol. 20(4), pp. 214-219 (in Russian). doi: http://dx.doi.org/:10.18821/1560-9588-2017-20-4-214-219
10. Fitch E., Harper E., Skorcheva I., Kurtz S.E., Blauvelt A. Pathophysiology of psoriasis: Recent advances on IL-23 and TH17 cytokines. *Curr. Rheumatol. Rep*, 2007, vol. 9, pp. 461-467 doi: 10.1007/s11926-007-0075-1
11. Wang A., Bai Y.P. Dendritic cells: The driver of psoriasis. *J. Dermatol,* 2020, vol. 47, pp. 104-113. doi: 10.1111/1346-8138.15184
12. Ogawa E., Sato Y., Minagawa A., Okuyama R. Pathogenesis of psoriasis and development of treatment. *J. Dermatol*, 2018, vol. 45, pp. 264-272. doi: 10.1111/1346-8138.14139
13. Psoriasis. Guidelines for the diagnosis and therapy of various forms of psoriasis and psoriatic arthritis. *Monograph. – St. Petersburg: Publishing House of DEAN*, 2014, p. 486
14. Terletsky O.V. Psoriasis. Differential diagnosis of "psoriasis-like" rare dermatoses. *Therapy. – St. Petersburg: DEAN*, 2007, p. 512.
15. Menter A., Korman N.J., Elmets C.A., Feldman S.R., Gelfand J.M., Gordon K.B., Gottlieb A., Koo J.Y.M., Lebwohl M., Lim H.W. Guidelines of care for the management of psoriasis and psoriatic arthritis. Section 5. Guidelines of care for the treatment of psoriasis with phototherapy and photochemotherapy. *J. Am. Acad. Dermatol*, 2010, vol. 62, pp. 114-135. doi: 10.1016/j.jaad.2009.08.026

ЛИТЕРАТУРА

1. Клинические рекомендации РФ 2023 «Псориаз»
2. Кубанова А.А., Кисина В.И., Блатун Л.А. Рациональная фармакотерапия заболеваний кожи и инфекций, передаваемых половым путем // Руководство для практикующих врачей. – 2005. – С. 882
3. Lowes M.A., Suárez-Fariñas M., Krueger J.G. Immunology of psoriasis // Annu. Rev. Immunol – 2014. – Vol. 32. – P. 227-255 doi: 10.1146/annurev-immunol-032713-120225
4. Усатине Р.П., Смит М.А., Мэйе Э.Дж. Атлассправочник практикующего врача. Дерматология. – 2012. – С. 536
5. Кей Ш.К., Стратигос Д.А., Лио П.А., Джонсон Р.А. Детская дерматология // Цветной атлас и справочник / Кей Шу-Мей Кэйн и др.; пер. с англ. под ред. О. Л. Иванова, А. Н. Львова. – М. : Издательство Панфилова ; БИНОМ. Лаборатория знаний. 2011. – С. 496
6. Kubanov A.A., Bogdanova E.V. Dermatovenereology of Russian Federation in 2020: Working Under a Pandemic // Vestnik Dermatologii i Venerologii. – 2021. – Vol. 97(4). – P. 08-32. doi: https://doi.org/10.25208/vdv1261
7. Zhang P., Wu M.X. A clinical review of phototherapy for psoriasis // Lasers Med. Sci. – 2018. – Vol. 33. – P. 173-180. doi: 10.1007/s10103-017-2360-1
8. Campbell J. Safe and effective use of phototherapy and photochemotherapy in the treatment of psoriasis // Br. J. Nurs. – 2020. – Vol. 29. – P. 547-552. doi: 10.12968/bjon.2020.29.10.547
9. Olisova O.Yu., Garanyan L.G. Epidemiology, etiopathogenesis, comorbidity in psoriasis – new facts // Russian Journal of Skin and Venereal Diseases (Rossiyskii Zhurnal Kozhnykh i Venericheskikh Boleznei). – 2017. – Vol. 20(4). – P. 214-219 (in Russian). doi: http://dx.doi.org/:10.18821/1560-9588-2017-20-4-214-219
10. Fitch E., Harper E., Skorcheva I., Kurtz S.E., Blauvelt A. Pathophysiology of psoriasis: Recent advances on IL-23 and TH17 cytokines // Curr. Rheumatol. Rep. – 2007. – Vol. 9. – P. 461-467 doi: 10.1007/s11926-007-0075-1
11. Wang A., Bai Y.P. Dendritic cells: The driver of psoriasis // J. Dermatol. – 2020. – Vol. 47. – P. 104-113. doi: 10.1111/1346-8138.15184
12. Ogawa E., Sato Y., Minagawa A., Okuyama R. Pathogenesis of psoriasis and development of treatment // J. Dermatol. – 2018. – Vol. 45. – P. 264-272. doi: 10.1111/1346-8138.14139
13. Псориаз. Руководство по диагностике и терапии разных форм псориаза и псоритического артрита // монография. – СПб.: Изд-во ДЕАН. – 2014. – С. 486
14. Терлецкий О.В. Псориаз. Дифференциальная диагностика «псориазоподобных» редких дерматозов // Терапия. – СПб.: ДЕАН. – 2007. – С. 512
15. Menter A., Korman N.J., Elmets C.A., Feldman S.R., Gelfand J.M., Gordon K.B., Gottlieb A., Koo J.Y.M., Lebwohl M., Lim H.W. Guidelines of care for the management of psoriasis and psoriatic arthritis. Section 5. Guidelines of care for the treatment of psoriasis with phototherapy and photochemotherapy // J. Am. Acad. Dermatol. – 2010. – Vol. 62. – P. 114-135. doi: 10.1016/j.jaad.2009.08.026

16. Kwiatkowski S., Knap B., Przystupski D., Saczko J., Kędzierska E., Knap-Czop K., Kotlińska J., Michel O., Kotowski K., Kulbacka J. Photodynamic therapy–Mechanisms, photosensitizers and combinations. *Biomed. Pharmacother*, 2018, vol. 106, pp. 1098-1107. doi: 10.1016/j.biopha.2018.07.049
17. Mehta D., Lim H.W. Ultraviolet B Phototherapy for Psoriasis: Review of Practical Guidelines. *Am. J. Clin. Dermatol*, 2016, vol. 17, pp. 125-133. doi: 10.1007/s40257-016-0176-6
18. Stern R.S. Psoralen and Ultraviolet A Light Therapy for Psoriasis. *N. Engl. J. Med*, 2007, vol. 357, pp. 682-690. doi: 10.1056/NEJMct072317
19. Reshetov I.V., Korenev S.V., Romanko Yu.S. Modern aspects of photodynamic therapy of basal cell skin cancer. *Biomedical Photonics*, 2022, vol. 11(3), pp. 35-39. doi: 10.24931/2413–9432–2022–11-3-35-39
20. Filonenko E.V., Ivanova-Radkevich V.I. Photodynamic therapy in the treatment of extramammary Paget disease. *Biomedical Photonics*, 2022, vol. 11(3), pp. 24-34 doi: 10.24931/2413–9432–2022–11-3-24-34
21. Filonenko E.V., Ivanova-Radkevich V.I. Photodynamic therapy in the treatment of patients with mycosis fungoides. *Biomedical Photonics*, 2022, vol. 11(1), pp. 27-36 (in Russian). doi: 10.24931/2413–9432–2022–11-1-27-36
22. Byun J.Y., Lee G.Y., Choi H.Y., Myung K.B., Choi Y.W. The expressions of TGF-β1and IL-10 in cultured fibroblasts after ALA-IPL photodynamic treatment. *Ann. Dermatol*, 2011, vol. 23, pp. 19-22. doi: 10.5021/ad.2011.23.1.19
23. Tandon Y.K., Yang M.F., Baron E.D. Role of photodynamic therapy in psoriasis: A brief review. *Photodermatol. Photoimmunol. Photomed*, 2008, vol. 24, pp. 222-230. doi: 10.1111/j.1600-0781.2008.00376.x
24. Kim J.Y., Kang H.Y., Lee E.S., Kim Y.C. Topical 5-aminolaevulinic acid photodynamic therapy for intractable palmoplantar psoriasis. *J. Dermatol*, 2007, vol. 34, pp. 37-40. doi: 10.1111/j.1346-8138.2007.00213.x
25. Schleyer V., Radakovic-Fijan S., Karrer S., Zwingers T., Tanew A., Landthaler M., Szeimies R.M. Disappointing results and low tolerability of photodynamic therapy with topical 5-aminolaevulinic acid in psoriasis. A randomized, double-blind phase I/II study. *J. Eur. Acad. Dermatol. Venereol,* 2006, vol. 20, pp. 823-828. doi: 10.1111/j.1468-3083.2006.01651.x
26. Radakovic-Fijan S., Blecha-Thalhammer U., Schleyer V., Szeimies R.M., Zwingers T., Hönigsmann H., Tanew A. Topical aminolaevulinic acid-based photodynamic therapy as a treatment option for psoriasis? Results of a randomized, observer-blinded study. *Br. J. Dermatol*, 2005, vol. 152, pp. 279-283. doi: 10.1111/j.1365-2133.2004.06363.x
27. Salah M., Samy N., Fadel M. Methylene blue mediated photodynamic therapy for resistant plaque psoriasis. *J. Drugs Dermatol*, 2009, vol. 8, pp. 42-49
28. Rook A.H., Wood G.S., Duvic M., Vonderheid E.C., Tobia A., Cabana B. A phase II placebo-controlled study of photodynamic therapy with topical hypericin and visible light irradiation in the treatment of cutaneous T-cell lymphoma and psoriasis. *J. Am. Acad. Dermatol*, 2010, vol. 63, pp. 984-990. doi: 10.1016/j.jaad.2010.02.039
29. Calzavara-Pinton P.G., Rossi M.T., Aronson E., Sala R., Arpaia N., Burtica E.C., Amerio P., Virgili A., Rossi R., Buggiani G. A retrospective analysis of real-life practice of off-label photodynamic therapy using methyl aminolevulinate (MAL-PDT) in 20 Italian dermatology departments. Part 1: Inflammatory and aesthetic indications. *Photochem. Photobiol. Sci*, 2013, vol. 12, pp. 148-157. doi: 10.1039/c2pp25124h
30. Shaheen M.A., Dakhli A.O., Hassen S.I. Comparison between the Efficacy of Intense Pulsed Light (I.P.L.) versus Photo-dynamic Therapy (P.D.T.) with Methylene-Blue in the Treatment of Psoriatic nails. *Photodiagnosis Photodyn Ther*, 2023, vol. 19, p. 103298 doi: 10.1016/j.pdpdt.2023.103298. Epub ahead of print. PMID: 36682430

16. Kwiatkowski S., Knap B., Przystupski D., Saczko J., Kędzierska E., Knap-Czop K., Kotlińska J., Michel O., Kotowski K., Kulbacka J. Photodynamic therapy–Mechanisms, photosensitizers and combinations // Biomed. Pharmacother. – 2018. – Vol. 106. – P. 1098-1107. doi: 10.1016/j.biopha.2018.07.049
17. Mehta D., Lim H.W. Ultraviolet B Phototherapy for Psoriasis: Review of Practical Guidelines // Am. J. Clin. Dermatol. – 2016. – Vol. 17. – P. 125-133. doi: 10.1007/s40257-016-0176-6
18. Stern R.S. Psoralen and Ultraviolet A Light Therapy for Psoriasis // N. Engl. J. Med. – 2007. – Vol. 357. – P. 682-690. doi: 10.1056/NEJMct072317
19. Reshetov I.V., Korenev S.V., Romanko Yu.S. Modern aspects of photodynamic therapy of basal cell skin cancer // Biomedical Photonics. – 2022. – Vol. 11(3). – P. 35-39. doi: 10.24931/2413–9432–2022–11-3-35-39
20. Filonenko E.V., Ivanova-Radkevich V.I. Photodynamic therapy in the treatment of extramammary Paget disease, Biomedical Photonics. – 2022. – Vol. 11(3). – P. 24-34 doi: 10.24931/2413–9432–2022–11-3-24-34
21. Filonenko E.V., Ivanova-Radkevich V.I. Photodynamic therapy in the treatment of patients with mycosis fungoides // Biomedical Photonics. – 2022. – Vol. 11(1). – P. 27-36 (in Russian). doi: 10.24931/2413–9432–2022–11-1-27-36
22. Byun J.Y., Lee G.Y., Choi H.Y., Myung K.B., Choi Y.W. The expressions of TGF-β1and IL-10 in cultured fibroblasts after ALA-IPL photodynamic treatment // Ann. Dermatol. – 2011. – Vol. 23. – P. 19-22. doi: 10.5021/ad.2011.23.1.19
23. Tandon Y.K., Yang M.F., Baron E.D. Role of photodynamic therapy in psoriasis: A brief review. Photodermatol. Photoimmunol. Photomed. 2008. – Vol. 24. – P. 222-230. doi: 10.1111/j.1600-0781.2008.00376.x
24. Kim J.Y., Kang H.Y., Lee E.S., Kim Y.C. Topical 5-aminolaevulinic acid photodynamic therapy for intractable palmoplantar psoriasis // J. Dermatol. – 2007. – Vol. 34. – P. 37-40. doi: 10.1111/j.1346-8138.2007.00213.x
25. Schleyer V., Radakovic-Fijan S., Karrer S., Zwingers T., Tanew A., Landthaler M., Szeimies R.M. Disappointing results and low tolerability of photodynamic therapy with topical 5-aminolaevulinic acid in psoriasis. A randomized, double-blind phase I/II study // J. Eur. Acad. Dermatol. Venereol. – 2006. – Vol. 20. – P. 823-828. doi: 10.1111/j.1468-3083.2006.01651.x
26. Radakovic-Fijan S., Blecha-Thalhammer U., Schleyer V., Szeimies R.M., Zwingers T., Hönigsmann H., Tanew A. Topical aminolaevulinic acid-based photodynamic therapy as a treatment option for psoriasis? Results of a randomized, observer-blinded study. Br. J. Dermatol. – 2005. – Vol. 152. – P. 279-283. doi: 10.1111/j.1365-2133.2004.06363.x
27. Salah M., Samy N., Fadel M. Methylene blue mediated photodynamic therapy for resistant plaque psoriasis // J. Drugs Dermatol. – 2009. – Vol. 8. – P.42-49
28. Rook A.H., Wood G.S., Duvic M., Vonderheid E.C., Tobia A., Cabana B. A phase II placebo-controlled study of photodynamic therapy with topical hypericin and visible light irradiation in the treatment of cutaneous T-cell lymphoma and psoriasis // J. Am. Acad. Dermatol. – 2010. – Vol. 63. – P. 984-990. doi: 10.1016/j.jaad.2010.02.039
29. Calzavara-Pinton P.G., Rossi M.T., Aronson E., Sala R., Arpaia N., Burtica E.C., Amerio P., Virgili A., Rossi R., Buggiani G. A retrospective analysis of real-life practice of off-label photodynamic therapy using methyl aminolevulinate (MAL-PDT) in 20 Italian dermatology departments. Part 1: Inflammatory and aesthetic indications // Photochem. Photobiol. Sci. – 2013. – Vol. 12. – P. 148-157. doi: 10.1039/c2pp25124h
30. Shaheen M.A., Dakhli A.O., Hassen S.I. Comparison between the Efficacy of Intense Pulsed Light (I.P.L.) versus Photo-dynamic Therapy (P.D.T.) with Methylene-Blue in the Treatment of Psoriatic nails // Photodiagnosis Photodyn Ther. – 2023. – Vol. 19. – P. 103298 doi: 10.1016/j.pdpdt.2023.103298. Epub ahead of print. PMID: 36682430

31. Tehranchinia Z., Barzkar N., Mohammad Riahi S., Khazan M. A comparison of the effects of clobetasol 0.05% and photodynamic therapy using aminolevulinic acid with red light in the treatment of severe nail psoriasis. *J. Lasers Med. Sci,* 2020, vol. 11, pp. 3-7 doi: 10.15171/jlms.2020.02
32. Carrenho L.Z.B., Moreira C.G., Vandresen C.C., Gomes R., Gonçalves A.G., Barreira S.M.W., Noseda M.D., Duarte M.E.R., Ducatti D.R.B., Dietrich M. Investigation of anti-inflammatory and anti-proliferative activities promoted by photoactivated cationic porphyrin. *Photodiagnosis Photodyn. Ther*, 2015, vol. 12, pp. 444-458. doi: 10.1016/j.pdpdt.2015.05.003
33. Liu H.Q., Wang Y.M., Li W.F., Li C., Jiang Z.H., Bao J., Wei J.F., Jin H.T., Wang A.P. Anti-Psoriasis Effects and Mechanisms of A-(8-Quinolinoxy) Zinc Phthalocyanine-Mediated Photodynamic Therapy. *Cell. Physiol. Biochem*, 2017, vol. 44, pp. 200-214. doi: 10.1159/000484647
34. Lin R.K., Venkatesan P., Yeh C.H., Chien C.M., Lin T.S., Lin C.C., Lin C.C., Lai P.S. Effective topical treatments using innovative NNO-tridentate vanadium(iv) complexes-mediated photodynamic therapy in a psoriasis-like mouse model. *J. Mater. Chem. B*, 2022, vol. 10, pp. 4759-4770. doi: 10.1039/D2TB00344A
35. Boehncke W.H., Sterry W., Kaufmann R. Treatment of psoriasis by topical photodynamic therapy with polychromatic light. *Lancet*, 1994, vol. 343, pp. 801. doi: 10.1016/S0140-6736(94)91883-X
36. Robinson D.J., Collins P., Stringer M.R., Vernon D.I., Stables G.I., Brown S.B., Sheehan-Dare R.A. Improved response of plaque psoriasis after multiple treatments with topical 5-aminolaevulinic acid photodynamic therapy. *Acta Derm. Venereol*, 1999, vol. 79, pp. 451-455. doi: 10.1080/000155599750009898
37. Collins P., Robinson D.J., Stringer M.R., Stables G.I., Sheehan-Dare R.A. The variable response of plaque psoriasis after a single treatment with topical 5-aminolaevulinic acid photodynamic therapy. *Br. J. Dermatol*, 1997, vol. 137, pp. 743-749. doi: 10.1111/j.1365-2133.1997.tb01111.x
38. Bissonnette R., Tremblay J.F., Juzenas P., Boushira M., Lui H. Systemic photodynamic therapy with aminolevulinic acid induces apoptosis in lesional T lymphocytes of psoriatic plaques. *J. Investig. Dermatol*, 2002, vol. 119, pp. 77-83. doi: 10.1046/j.1523-1747.2002.01827.x
39. Fransson J., Ros A.M. Clinical and immunohistochemical evaluation of psoriatic plaques treated with topical 5-aminolaevulinic acid photodynamic therapy. Photodermatol. *Photoimmunol. Photomed*, 2005, vol. 21, pp. 326-332. doi: 10.1111/j.1600-0781.2005.00182.x
40. Smits T., Kleinpenning M.M., Van Erp P.E.J., Van De Kerkhof P.C.M., Gerritsen M.J.P. A placebo-controlled randomized study on the clinical effectiveness, immunohistochemical changes and protoporphyrin IX accumulation in fractionated 5-aminolaevulinic acid-photodynamic therapy in patients with psoriasis. *Br. J. Dermatol*, 2006, vol. 155, pp. 429-436 doi: 10.1111/j.1365-2133.2006.07290.x

31. Tehranchinia Z., Barzkar N., Mohammad Riahi S., Khazan M. A comparison of the effects of clobetasol 0.05% and photodynamic therapy using aminolevulinic acid with red light in the treatment of severe nail psoriasis // J. Lasers Med. Sci. – 2020. – Vol. 11. – P. 3-7 doi: 10.15171/jlms.2020.02
32. Carrenho L.Z.B., Moreira C.G., Vandresen C.C., Gomes R., Gonçalves A.G., Barreira S.M.W., Noseda M.D., Duarte M.E.R., Ducatti D.R.B., Dietrich M. Investigation of anti-inflammatory and anti-proliferative activities promoted by photoactivated cationic porphyrin // Photodiagnosis Photodyn. Ther. – 2015. – Vol. 12. – P. 444-458. doi: 10.1016/j.pdpdt.2015.05.003
33. Liu H.Q., Wang Y.M., Li W.F., Li C., Jiang Z.H., Bao J., Wei J.F., Jin H.T., Wang A.P. Anti-Psoriasis Effects and Mechanisms of A-(8-Quinolinoxy) Zinc Phthalocyanine-Mediated Photodynamic Therapy // Cell. Physiol. Biochem. – 2017. – Vol. 44. – P. 200-214. doi: 10.1159/000484647
34. Lin R.K., Venkatesan P., Yeh C.H., Chien C.M., Lin T.S., Lin C.C., Lin C.C., Lai P.S. Effective topical treatments using innovative NNO-tridentate vanadium(iv) complexes-mediated photodynamic therapy in a psoriasis-like mouse model // J. Mater. Chem. B. – 2022. – Vol. 10. – P. 4759-4770 doi: 10.1039/D2TB00344A
35. Boehncke W.H., Sterry W., Kaufmann R. Treatment of psoriasis by topical photodynamic therapy with polychromatic light // Lancet. – 1994. – Vol. 343. – P. 801 doi: 10.1016/S0140-6736(94)91883-X
36. Robinson D.J., Collins P., Stringer M.R., Vernon D.I., Stables G.I., Brown S.B., Sheehan-Dare R.A. Improved response of plaque psoriasis after multiple treatments with topical 5-aminolaevulinic acid photodynamic therapy // Acta Derm. Venereol. – 1999. – Vol. 79. – P. 451-455 doi: 10.1080/000155599750009898
37. Collins P., Robinson D.J., Stringer M.R., Stables G.I., Sheehan-Dare R.A. The variable response of plaque psoriasis after a single treatment with topical 5-aminolaevulinic acid photodynamic therapy // Br. J. Dermatol. – 1997. – Vol. 137. – P. 743-749 doi: 10.1111/j.1365-2133.1997.tb01111.x
38. Bissonnette R., Tremblay J.F., Juzenas P., Boushira M., Lui H. Systemic photodynamic therapy with aminolevulinic acid induces apoptosis in lesional T lymphocytes of psoriatic plaques // J. Investig. Dermatol. – 2002. – Vol. 119. – P. 77-83. doi: 10.1046/j.1523-1747.2002.01827.x
39. Fransson J., Ros A.M. Clinical and immunohistochemical evaluation of psoriatic plaques treated with topical 5-aminolaevulinic acid photodynamic therapy // Photodermatol. Photoimmunol. Photomed. – 2005. – Vol. 21. – P. 326-332. doi: 10.1111/j.1600-0781.2005.00182.x
40. Smits T., Kleinpenning M.M., Van Erp P.E.J., Van De Kerkhof P.C.M., Gerritsen M.J.P. A placebo-controlled randomized study on the clinical effectiveness, immunohistochemical changes and protoporphyrin IX accumulation in fractionated 5-aminolaevulinic acid-photodynamic therapy in patients with psoriasis // Br. J. Dermatol. – 2006. – Vol. 155. – P. 429-436 doi: 10.1111/j.1365-2133.2006.07290.x

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