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ORIGINAL ARTICLES

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ОРИГИНАЛЬНЫЕ СТАТЬИ

ОБЗОРЫ ЛИТЕРАТУРЫ

ORIGINAL ARTICLES

EFFECTIVENESS OF PURPLE LED FOR INACTIVATION OF BACILLUS SUBTILIS AND ESCHERICHIA COLI BACTERIA IN IN VITRO STERILIZERS

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Abstract

Bacteria are inactivated using a technique called photodynamic inactivation, which combines light with a photosensitizer with the right spectrum. The objective of this study is to ascertain the efficiency of purple LEDs for photoinactivating *Bacillus subtilis* and *Escherichia coli* bacteria as well as the ideal purple LED exposure energy density. This study technique involves exposing bacteria to purple LED radiation. Two elements of variation are used during irradiation. The first variation is the illumination variation at distances of 3 cm, 6 cm, 9 cm, and 12 cm. The second variation involves changing the amount of radiation for 30, 60, 90, and 120 minutes. The Total Plate Count (TPC) method was used to count the number of colonies. Statistical tests were utilized in data analysis, namely the One Way Anova test (analysis of variance). The results of this study indicated that 395 nm purple LED irradiation caused a decrease in Log CFU/mL of *Bacillus subtilis* and *Escherichia coli* bacteria. Inactivation of *Bacillus subtilis* bacteria showed a higher mortality percentage than *Escherichia coli* bacteria. Changes in other irradiation distances also showed a higher percentage of death for *Bacillus subtilis* bacteria than *Escherichia coli* bacteria. The highest percentage of death was 98.5% for Bacillus subtilis bacteria and 94.3% for *Escherichia coli* bacteria at position C with an irradiation distance of 3 cm and an energy density of 524 J/cm^2 with an LED exposure time of 120 minutes. This shows that the percentage of death of bacteria *Bacillus subtilis* and *Escherichia coli* increased with increasing doses of LED energy with the greatest percentage of death in Gram-positive bacteria *Bacillus subtilis*.

Key words: health security, photodynamic inactivation, purple LED, *Bacillus subtilis, Escherichia coli.*

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ЭФФЕКТИВНОСТЬ IN VITRO ИНАКТИВАЦИИ БАКТЕРИЙ BACILLUS SUBTILIS И ESCHERICHIA COLI В СТЕРИЛИЗАТОРАХ С ИСПОЛЬЗОВАНИЕМ ОБЛУЧЕНИЯ В ФИОЛЕТОВОЙ ОБЛАСТИ

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Резюме

Инактивация бактерий может быть выполнена с использованием метода, называемого фотодинамической инактивацией, в основе которого лежит активация фотосенсибилизатора светом определенного спектра. Целью данного исследования является определение эффективности светодиодов с излучением в фиолетовой области спектра для фотоинактивации бактерий Bacillus subtilis и Escherichia coli, а также определение оптимальной плотности энергии воздействия. При облучении были использованы два изменяемых параметра. Первый параметр — это расстояние от источника облучения до облучаемой поверхности (3 см, 6 см, 9 см и 12 см). Второй параметр – время облучения (30, 60, 90 и 120 мин). Для подсчета количества колоний использовали метод общего подсчета чашек (Total Plate Count). При анализе данных использовали статистические тесты, а именно тест One Way Anova (дисперсионный анализ). Результаты этого исследования показали, что светодиодное излучение в фиолетовой области спектра с длиной волны 395 нм вызывало снижение log КОЕ/мл бактерий *Bacillus subtilis* и *Escherichia coli*. Воздействие на бактерии *Bacillus subtilis* показало более высокий процент смертности, чем для бактерий *Escherichia coli*. Лучшие результаты были получены при расстоянии до источника облучения 3 см, плотности энергии 524 Дж/см^2, и времени воздействия светодиода 120 мин. В этом режиме было инактивировано 98,5% бактерий *Bacillus subtilis* и 94,3% бактерий *Escherichia coli*.

Ключевые слова: безопасность, фотодинамическая инактивация, фиолетовый светодиод, *Bacillus subtilis, Escherichia coli*.

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Introduction

The goal of sterilizing health services is to achieve the best/highest level of individual/community health by effort, work, or health-related activities. In hospitals and other healthcare facilities in Indonesia, infection and sepsis continue to be among the leading causes of mortality and morbidity. The spread of infections and diseases can have a negative impact on the health of employees, patients, and the medical procedures performed at healthcare facilities. The presence of contaminating bacteria as an infection source has a significant impact in settings that should be kept sterile, including operating rooms, laboratories, and existing medical equipment. *Bacillus subtilis* is a common contamination microorganism detected in medical equipment. *Escherichia coli* is the bacteria that is frequently discovered in food. These bacteria's spores are also agents that contribute to food spoiling, operate as disease vectors, and can alter food quality, which makes them a serious problem for the food industry [1].

Additionally, the cleanliness of the food we eat has an impact on our health. The application of cleanliness, safety, comfort, and regularity which decreases or even avoids the possibility of contamination is the primary principle of food sanitation. using food sanitation to regulate the usage of raw materials, processing locations, and auxiliary equipment [2]. It must be ensured that the tools used are clean before they may be used to prepare and serve food.

All biological forms are eliminated during sterilization. From a microbiological perspective, a thing is free of living microorganisms if it is sterile. Among all living things, bacterial spores are the most resistant to sterilization. The quantity and kind of microorganisms, the degree and type of contamination by other substances, and whether or not microorganisms are present on the device all affect how effectively a device is sterilized [3]. Dental instruments and other items that come into contact with blood or bodily tissue must be sterilized. Dry heat sterilization is one of the most popular sterilization processes. The drawbacks of the dry heat sterilization approach include the lengthier sterilization time, the slow and uneven material penetration, the need for an oven and a constant power source, the inability to disinfect plastic and rubber devices, and the high cost of the sterilizing equipment. In order to effectively inactivate contaminating bacteria, a different approach including photodynamic inactivation is therefore required. Previous studies have been conducted to see the effectiveness of light for inactivating bacteria and fungi [4, 5].

Photoinactivation is the process of preventing cellular metabolism due to cytoplasmic membrane damage brought on by reactive oxygen in lipids and proteins [6]. The membrane transport system in the bacterial cell is either inactive or undergoes cell lysis as a result of reactive oxygen. The light source, the photosensitizer as a substance, and the free radicals that lead to cell inactivation are the three primary causes of photodynamic' success. Endogenous porphyrins, which some bacteria naturally create and which are photosensitizers and light-absorbing compounds (sensitive to light). Every porphyrin molecule has the capacity to absorb light of a specific wavelength [7]. Bacterial cells will be photo inactivated when the right combination of light and photosensitizer is used [8]. A photosensitization mechanism, such as the absorption of light by porphyrins, triggers reactions in the substrate to begin the photoinactivation process. External photosensitizers from various materials such as chemicals [9], drugs [10], organic/natural materials [11] and metals [12] can be added to increase the effectiveness of photoinactivation. The type and number of

photosensitizers, which act as light-absorbing molecules, determine this photosensitization.

The Light-emitting diode (LED), a complicated semiconductor that can convert electrical energy into light, has the advantage of only releasing a little amount of heat in the light it generates. It is one of the light sources that has a porphyrin absorption spectrum range of the photosensitizer type. The porphyrin absorption spectral region of the photosensitizer type is present in LED light sources. Additionally, because LEDs only generate a minimal amount of heat in the light they provide, they are superior to conventional light sources for the phototherapy process [13]. Previous studies demonstrated the ability of LEDs to inactivate bacteria [5, 14, 15]

The result study previously showed that a purple LED with a wavelength of 408.6 nm, energy of 61.2 joules, and a photoinactivation effect of 42.11% was the best light source for inactivating *S. mutans* [14]. In another investigation the visible light with a wavelength of 405 nm used to test the susceptibility of *Bacillus* and *Clostridium endospores* [16]. Another study with light 405 nm showed endospores can greatly be affected by light's bactericidal effects [17]. In contrast, the inactivation of the *Listeria monocytogenes bacteria* utilizing high-intensity light with a 405 nm wavelength [18]. The findings demonstrated that optimum inactivation is induced by exposure to the 400–450 nm wavelength range at a rather high dose level (750 J/cm^2). Longer than 450 nm exposure does not result in substantial inactivation. The most efficient wavelength for inactivating *L. monocytogenes* is 405 nm of light, according to an analysis with a 10 nm bandwidth between 400 and 450 nm. These findings informed the choice of a shorter wavelength, higher intensity purple LED light source for this research. In this study, *Bacillus subtilis* and *Escherichia coli* bacteria will be photo inactivated in vitro using the best purple LED exposure energy density and effectiveness.

Materials and methods

Bacterial Culture

The bacterial strain, *Bacillus subtilis* ATCC 9466 and *Escherichia coli* ATCC 25922 was inoculated from Tryptone Soy Agar (Oxoid, UK) and taken on Tryptone Soy Broth sterile (Oxoid, UK). The culture of bacteria was incubated at 37°C until bacterial colonies reached ~108 CFU/mL or 1.0 McFarland Standard.

Purple LED Exposure

Purple LEDs with a peak wavelength of 395 nm were exposed to *Bacillus subtilis* and *Escherichia coli* bacteria. The instruments used in this study were purple LEDs 395 nm arranged on 10x10 pieces of PCB and assembled in an acrylic box with a volume of 15x15x15 cm^3 and controlled by a microcontroller. The instrument is equipped with a display of time (minutes), PWM (%), and irradiation temperature (°C). The process of irradiating the LEDs on the samples was carried out in various positions, namely positions A, B, C, D, and E according to Fig. 1. Treatment of bacteria was carried out at various distances, namely at a distance of 3 cm, 6 cm, 9 cm, and 12 cm and time variations exposure for 30 minutes, 60 minutes, 90 minutes and 120 minutes. Table 1 shows the average values of the measurement data for the intensity of a 395 nm purple LED at a distance of 3 cm, 6 cm, 9 cm and 12 cm.

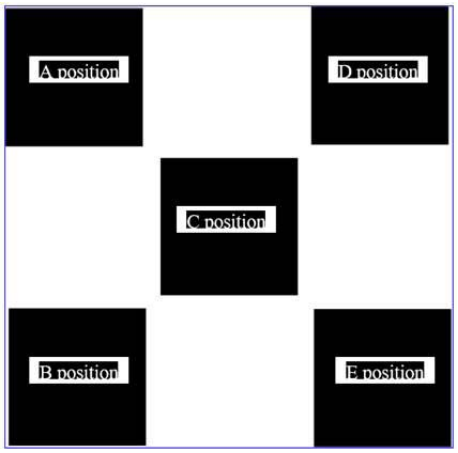

Рис. 1. Положение образца.
Fig. 1. Position of sample.

Table 1
The average values of the measurement data for the intensity of a 395 nm purple LED at a distance of 3 cm, 6 cm, 9 cm and 12 cm
Таблица 1
Средние значения данных измерений интенсивности излучения фиолетового светодиода с длиной волны 395 нм на расстоянии 3 см, 6 см, 9 см и 12 см

Distance, cm	A Position, $\frac{mW}{cm^2}$	B Position, $\frac{mW}{cm^2}$	C Position, $\frac{mW}{cm^2}$	D Position, $\frac{mW}{cm^2}$	E Position, $\frac{mW}{cm^2}$
3	49.4	59.6	89.0	54.1	54.5
6	39.2	41.6	67.6	41.2	40.3
9	27.0	27.0	55.1	24.9	26.9
12	15.6	20.5	35.0	23.0	12.9

Data analysis

Using the total plate count approach, the number of bacterial colonies that were growing was counted. The following formula is used to determine the percentage decrease in the number of bacterial colonies:

$$\%death = \left| \frac{\sum treatments - \sum control}{\sum control} \right| x\,100\,\%$$

The One-Way ANOVA test was used as the statistical analysis (analysis of variance). The purpose of this test is to identify any variations in the outcomes of each treatment group.

Results and discussion

Bacillus subtilis and *Escherichia coli* bacteria were exposed to purple LEDs at varying distances of 3, 6, and 9 cm for periods of 30, 60, 90, and 120 minutes with a power width modulation (PWM) value of 100%. Fig.1 and 2 showed *Bacillus subtilis* and *Escherichia coli* viability after LED exposure to (a) position A, (b) position B, (c) position C, (d) position D, (e) position E.

Based on research data, power density and energy density values are obtained at each position. The position that has the greatest power density is at position C so that the energy density value has the greatest value as well. The power density at positions A, B, D, E have almost the same

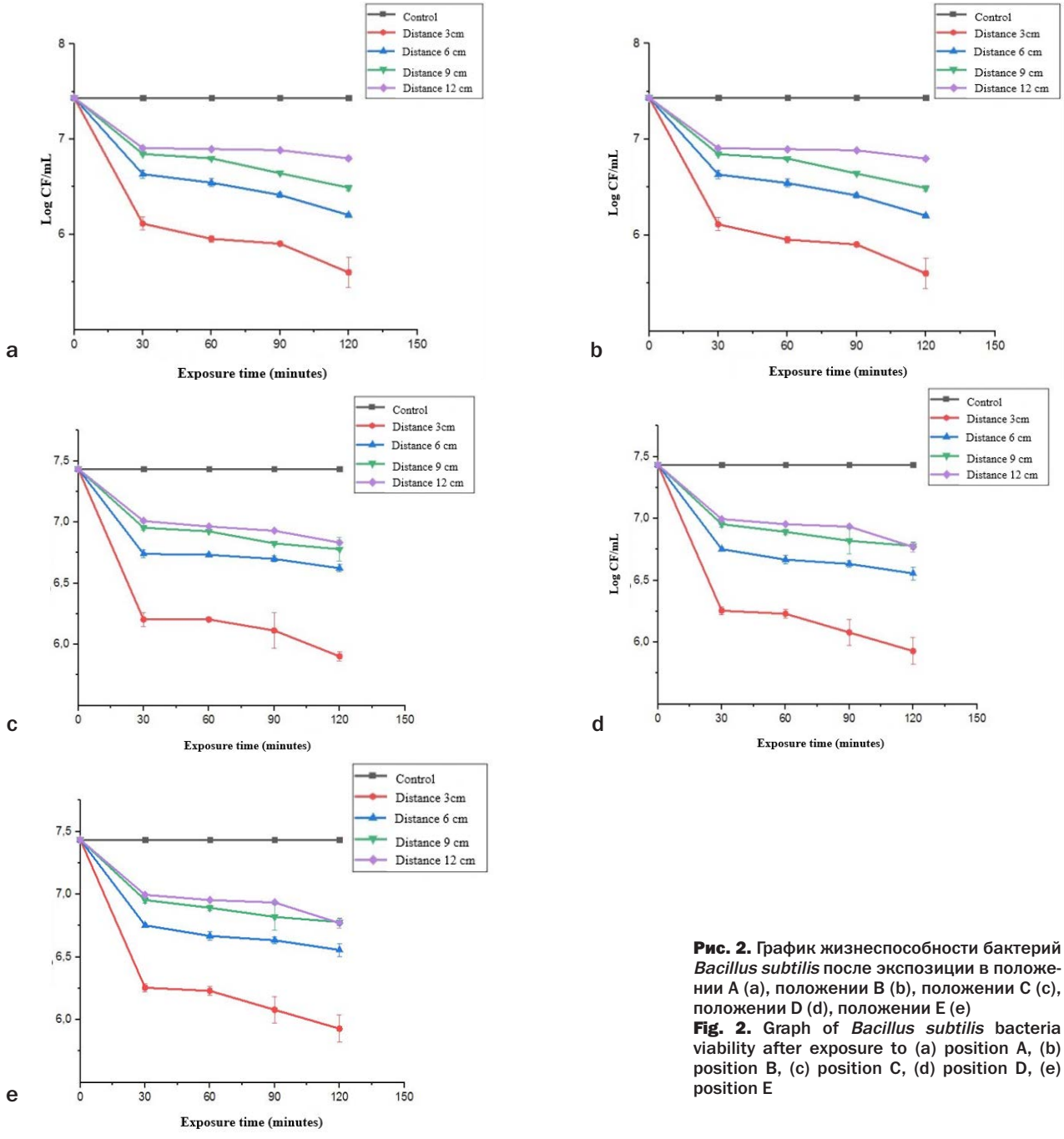

Рис. 2. График жизнеспособности бактерий *Bacillus subtilis* после экспозиции в положении A (a), положении B (b), положении C (c), положении D (d), положении E (e)

Fig. 2. Graph of *Bacillus subtilis* bacteria viability after exposure to (a) position A, (b) position B, (c) position C, (d) position D, (e) position E

values so that the energy density or dose at positions A, B, D, and E also have almost the same values. Therefore, the value of the percentage of deaths in these positions has almost the same value. One Way Anova test findings with a significance or probability (p) value of 0.00 indicate that there is a significant difference between the treatments in this study.

The results of this study indicated that 395 nm purple LED irradiation caused a decrease in Log CFU/mL of *Bacillus subtilis* and *Escherichia coli* bacteria. Inactivation of *Bacillus subtilis* bacteria showed a higher mortality percentage than *Escherichia coli* bacteria. Changes in other irradiation distances also showed a higher percentage of death for *Bacillus subtilis* bacteria than *Escherichia coli* bacteria. The highest percentage of death was 98.5% for *Bacillus subtilis* bacteria and 94.3% for *Escherichia coli* bacteria at position C with an irradiation distance of 3 cm and an energy density of 524 J/cm^2 with an LED exposure time of 120 minutes. This shows that the percentage of death of bacteria *Bacillus subtilis* and *Escherichia coli* increased with increasing doses of LED energy with the greatest percentage of death in Gram-positive bacteria *Bacillus subtilis.*

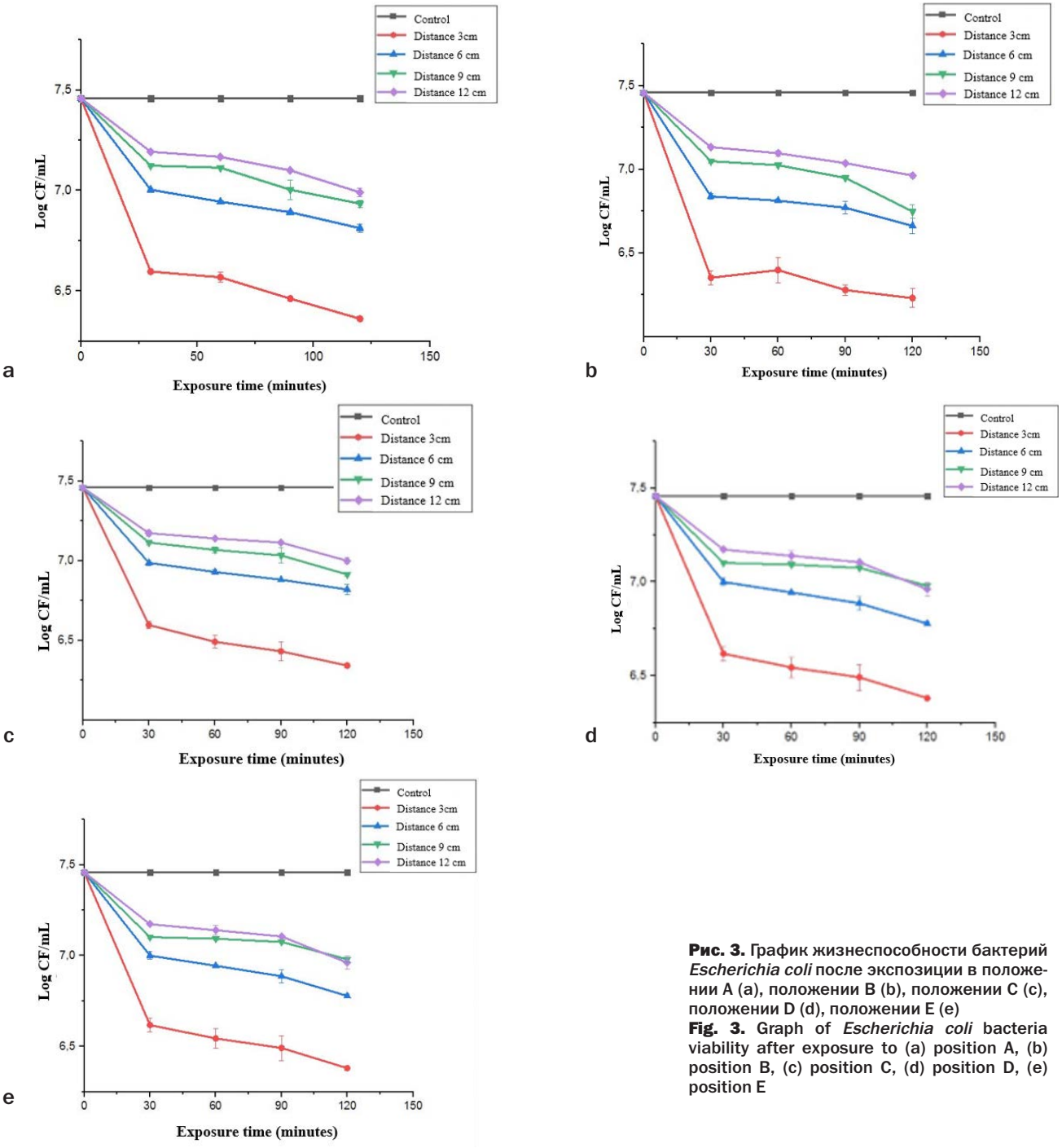

Рис. 3. График жизнеспособности бактерий *Escherichia coli* после экспозиции в положении A (a), положении B (b), положении C (c), положении D (d), положении E (e)
Fig. 3. Graph of *Escherichia coli* bacteria viability after exposure to (a) position A, (b) position B, (c) position C, (d) position D, (e) position E

Bacteria are photo inactivated by the process of photodynamic inactivation (PDI), which is regulated by oxygen, photosensitizer material, and light source. Photoinactivation is the process of preventing cellular metabolism due to cytoplasmic membrane damage brought on by reactive oxygen in lipids and proteins. The membrane transport system in the bacterial cell is either inactive or undergoes cell lysis as a result of reactive oxygen. A 395 nm-wavelength purple LED light source was employed in the study. *Bacillus subtilis* and *Escherichia coli* bacteria's absorption spectrum range is taken into account while adjusting the wavelength.

Power density and energy density numbers are determined for each place based on research data. Position C has the highest power density, which also means that this position also has the highest energy density rating. Because the power density at points A, B, D, and E is almost identical, the energy density or dose at these locations is almost identical as well. As a result, the percentage of fatalities in these positions is practically the same.

A photophysical mechanism underlies the photoinactivation process that happens in *Bacillus subtilis* and *Escherichia coli*. *Bacterial cells* will be photo inactivated when the right combination of light and photosensitizer is used. A photosensitization mechanism, such as light absorption by porphyrins, triggers reactions in the substrate to produce radical oxygen species (ROS) [19]. First step is absorption of photon energy by endogenous porphyrin (10^{-15} s) followed by porphyrin molecule excitation. The excitation is generally achieved via a one photon transition between the ground state and a singlet excited state. Intersystem crossing generates the sensitizer triplet state. The lifetime of this state is longer (ms) so it will react with biology subtract in types I and II photochemistry mechanisms. A Type I mechanism involves hydrogen-atom abstraction or electron-transfer between the excited porphyrin and a substrate, yielding free radicals [20]. These radicals can react with oxygen to form an active oxygen species such as the superoxide radical anion. In a Type II mechanism, singlet oxygen is generated via an energy transfer process during a collision of the excited porphyrin with triplet oxygen. Effect of ROS is damage to the cytoplasmic membrane, allowing leakage of cellular contents or inactivation of membrane transport systems and enzymes that caused peroxidation in lipid and membrane proteins and cell lysis [21, 22].

Bacillus subtilis and *Escherichia coli* are Gram positive and Gram-negative bacteria. Differences of PDT inactivation effect on Gram-positive and Gram-negative lies in the structure of the cell wall [23]. On the outside wall of Gram-positive bacteria with a thickness of 15-80 nm consisting of 100 layers peptidoglycan associated with lipoprotein that binds to the outer membrane and the peptidoglycan teichuronic acid negatively charged relatively porous. The outer membrane of Gram-negative bacteria consisting of lipopolysaccharide, phospholipids, and lipoproteins. outer membrane serves as a barrier against the damaging effects of the outside of the cell and has a permeability to certain molecules [24]. The outer membrane forms an effective barrier permeability. Photochemical reactions type I operative for Gram (+) and reaction type II operative to Gram (-). 90% of singlet oxygen reacts to the cell lipid bilayer and proteins associated with the membrane transport system, lipid and protein peroxidation occurs causing damage to the cytoplasmic membrane and protein denaturation resulting in inactivation of the membrane transport system, interference with cell wall synthesis and the emergence of a multilamellar structure on the side of the cell divider. which cleaves and leaks potassium ions and then cell lysis occurs [23].

Bacillus subtilis and *Escherichia coli* undergo photoinactivation by a photophysical mechanism. When the correct amount of light and photosensitizer are utilized, bacterial cells will be photo inactivated. The photoinactivation process starts when processes in the substrate are triggered by a photosensitization mechanism, such as light absorption by porphyrins. Reactive oxygen is produced during this process, which causes the bacterial cell to lyse or disables the membrane transport system. While others blame radicals like HO for the destruction, many authors mistakenly assume that O_2 is the sole species that matters when it comes to bacterial PDI. According to a theory, Gram-positive bacteria are more sensitive to O_2, whereas Gram-negative bacteria are more sensitive to HO. Variations in the amount that PS binds to the bacteria's microenvironment or the amount of NaN3 that enters the bacterial cell walls may also contribute to variations in NaN_3 inhibition. By employing S. verbascifolium as the PS, we found that the PDI reaction was oxygen-dependent, considerably inhibited by sodium azide, and only marginally inhibited by mannitol. Through the use of type II and type I reactions, respectively, this demonstrated the dependency on singlet oxygen and, to a lesser extent, hydroxyl radicals.

Conclusion

The results of this study indicated that 395 nm purple LED irradiation caused a decrease in Log CFU/mL of *Bacillus subtilis* and *Escherichia coli* bacteria. Inactivation of *Bacillus subtilis* bacteria showed a higher mortality percentage than *Escherichia coli* bacteria. Changes in other irradiation distances also showed a higher percentage of death for *Bacillus subtilis* bacteria than *Escherichia coli* bacteria. The highest percentage of death was 98.5% for *Bacillus subtilis* bacteria and 94.3% for *Escherichia coli* bacteria at position C with an irradiation distance of 3 cm and an energy density of 524 J/cm^2 with an LED exposure time of 120 minutes. This shows that the percentage of death of bacteria *Bacillus subtilis* and *Escherichia coli* increased with increasing doses of LED energy with the greatest percentage of death in Gram-positive bacteria *Bacillus subtilis*.

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DEVELOPMENT OF A METHOD FOR ASSESSING THE DEPTH OF PENETRATION OF ETHOSOMES WITH METHYLENE BLUE INTO THE SKIN DURING APPLICATION AND PHOTODYNAMIC EXPOSURE

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Abstract

A wide range of literature sources report on the potential benefits of transdermal drug delivery. Among these advantages, the following are distinguished – minimal injury, reduction of side effects, and prevention of degradation or metabolism in the gastrointestinal tract or liver. However, transdermal delivery of most molecules often excludes due to the barrier function of the skin, which prevents the penetration of exogenous substances. To overcome this barrier and increase skin absorption, ethosomal complexes use, by means penetration into the deep layers of the skin and/or systemic circulation is possible. This work devotes to the development of a non-invasive method for assessing the depth of penetration by ethosomes with methylene blue (MB) into the skin during application and photodynamic exposure. MB as photosensitizer (PS) was chosen, since there are a sufficient number of publications on its positive effect on the restoration of the cell's respiratory chain of various organs and therefore the restoration of their metabolism. Besides MB has proven to be an effective PS, destructed pathogenic microbes and viruses, including SARS-CoV-2. However, for more effective Covid-19 therapy and antibiotic-resistant microbial diseases, the penetration of MB into the vascular system of the epidermis or mucous tissue is required. Nowadays, the existing methods for assessing the penetration depth of PS are time consuming and require the use of animal skin or model samples. The LESA-01 BIOSPEC system with specially designed optical adapters that allow assessing the drug fluorescence intensity on skin surface and at a depth of up to 2 mm in the investigation was used.

Keywords: ethosomes, transdermal drug delivery, methylene blue, penetration depth, effectivethickness of the drug layer.

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РАЗРАБОТКА МЕТОДА ОЦЕНКИ ГЛУБИНЫ ПРОНИКНОВЕНИЯ ЭТОСОМ С МЕТИЛЕНОВЫМ СИНИМ В КОЖУ ПРИ АППЛИКАЦИОННОМ ПРИМЕНЕНИИ И ФОТОДИНАМИЧЕСКИМ ВОЗДЕЙСТВИИ

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Резюме

В широком спектре литературных источников сообщается о потенциальных преимуществах трансдермальной доставки лекарственных веществ. Среди данных преимуществ выделяют следующие – минимальная травматичность, снижение побочных эффектов, предотвращение деградации или метаболизма в желудочно-кишечном тракте или печени. Однако трансдермальная доставка большинства молекул часто исключается из-за барьерной функции кожи, которая препятствует проникновению экзогенных веществ. Для преодоления данного барьера и увеличения кожного поглощения могут быть использованы этосомальные комплексы, с помощью которых возможно проникновение в глубокие слои кожи и/или системное кровообращение. Данная работа посвящена разработке неинвазивного метода оценки глубины проникновения этосом с метиленовым синим в кожу при аппликационном применении и фотодинамическом воздействии. Именно метиленовый синий был выбранв качестве фотосенсибилизатора (ФС) в работе, поскольку имеется достаточное количество публикаций о его положительном влиянии на восстановление дыхательной цепи клеток различных органов и, тем самым восстановлении их метаболизма. Кроме того, метиленовый синий проявил себя как эффективный ФС, разрушающий патогенные микробы и вирусы, в том числе вирус SARS-CoV-2. Однако для более эффективной терапии Covid-19 и антибиотикорезистентных микробных заболеваний требуется проникновение метиленовый синий в сосудистую систему эпидермиса или слизистой ткани. Наданный момент существующие методы оценки глубины проникновения фотосенсибилизаторов являются трудоёмкими и требуют использования кожи животных или модельных образцов. В работе была использована система ЛЭСА-01 БИОСПЕК со специально разработанными оптическими адапторами, позволяющими оценивать интенсивность флуоресценции препарата наповерхности кожи и на глубине до 2 мм.

Ключевые слова: этосомы, трансдермальная доставка лекарств, метиленовый синий, глубина проникновения, вирусы, микробы.

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Introduction

In recent decades, there has been a diverse and widespread use of lasers in dermatology and cosmetology. Laser methods to correct age-related changes and various skin pathologies are used [6]. Such a desire for laser therapies has led to the development of highly effective, minimally invasive and sparing methods of treatment [1], one of which is photodynamic therapy, which in the treatment of various skin diseases is used. In addition to cancerous and precancerous skin changes, PDT for cosmetic purposes in photo-rejuvenation is used [2]. Improvement of the skin condition during photoaging, prevention of actinic keratoses, the possibility of repeated procedures and a limited number of side effects make the PDT procedure very promising for skin rejuvenation [3]. One of the photosensitizers in PDT is methylene blue (MB). Several clinical studies indicate the effectiveness of MB in the treatment of basal cell carcinoma, Kaposi's sarcoma, melanoma, viral and fungal infections are used [4]. The authors [5] also note the antioxidant effect of MB and prove that its independent use can effectively protect the skin from oxidative stress and slow down skin aging.

The MB photosensitizer differs from a number of other photosensitizers, since during photoexcitation it oxidizes NAD(P)H, which is localized in the mitochondrial matrix, which defines MB as a photosensitizer of mitochondrial action [4,6]. In fact, targeting mitochondria is an important subject of research in PDT, since damage to mitochondria can induce an apoptotic cascade [4,7,8].

Brief description of the structure, functions and ways of penetration into the skin

The skin is the outer and largest organ of the human body with a complex structure. The skin performs a protective function and acts as a barrier to the penetration of exogenous substances from the external environment into the body. Stratum corneum (SC) is a "brick" organization consisting of corneocytes embedded in the lipid domain. The strengthening of cell walls is due to the presence of covalently bound lipids and cross-linked proteins, and the connection with neighboring cells occurs through desmosomes. Directly under the SC is a viable epidermis with keratinocytes, formed in the basal layer of the epidermis. Then there is a slow upward migration of these cells to the surface of the skin. Melanocytes, Langerhans cells, migrating macrophages and lymphocytes in the epidermis were also found. Under the epidermis there is a dermis containing structured collagen and elastin fibers. The epidermis and dermis perform an important function in the process of percutaneous absorption. The hypoderm under the dermis is located and is a layer of subcutaneous adipose tissue, provides the main food supply, physical protection and thermal insulation [9,10]. For the vast majority of penetrants, diffusion through SC associated with an obstacle and a restriction on the penetration rate. Since SC consists of dead cells where there is no metabolic activity, the penetration process occurs in a passive way. Such an obstacle to penetration with the composition and structure of the SC itself is related [11]. At the same

time, the only continuous region in SC is the lipid domain mainly consisting of ceramides, free fatty acids, cholesterol and cholesterol esters. A distinctive feature is the difference from other biological membranes, which consist mainly of phospholipids. Such a unique composition of SC prevents the penetration of ionic, high-polar substances and macromolecules. Highly polypophilic molecules, passing through the SC, do not easily diffuse into the hydrophilic epidermis and dermis [9].

There are three potential methods of penetration to deep layers by application: the intercellular pathway, the transcellular pathway and the accessory pathway (Fig. 1) [12]. Since sweat glands and hair follicles occupy only 0.1% of the total body surface, the accessory pathway does not contribute much to the penetration of medicinal substances [9,12]. Although, when the penetration of slowly diffusing compounds and substances with high molecular weight, such as nanoparticles, occurs, the accessory pathways may have an important role [13]. However, it is generally assumed that the intercellular pathway is the main one for the penetration of most molecules [14].

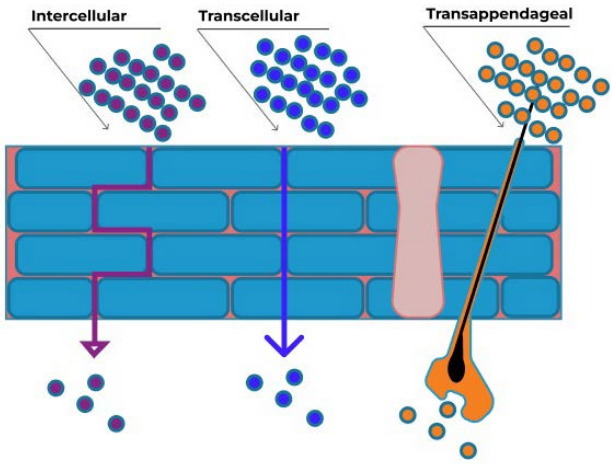


Рис. 1. Возможные пути доставки лекарства сквозь роговой слой кожи
Fig. 1. Impossible routes of drug delivery trough stratum corneum

To achieve the therapeutic amount of the drug in the deep layers of the skin and systemic circulation, appropriate penetration enhancers can be used, which affect the properties of the skin barrier and/or penetrate. Four main methods of enhancing penetration through the skin are most often considered: microneedle delivery, the use of electrical impulses, chemical reinforcement and the use of innovative vesicular carriers [9].

Innovative vesicular carries

Liposomes as a drug penetration enhancer during topical application of the drug by Mezei about two decades ago were first investigated [15,16]. According to further studies, it was shown that such rigid particles increase accumulation in the upper layers of the skin and do not lead to an increase of medicinal substance in the deep layers of the skin [17,18]. Therefore, efforts to synthesize lipid vesicular systems that can facilitate the penetration of the drug into the underlying layers of the skin and allow transdermal absorption were made [19,20]. Innovative vesicular carriers should include ethosomes. The main components of the ethosomes are phospholipids, ethanol (20-45%) and water. In special cases, propylene glycol (PG), carbopol and isopropyl alcohol to the composition are added [21].

Eggs, soy, polysynthetic and synthetic products can be used as a source of phospholipids. The high concentration of alcohol in the composition provides a soft shape and allows you to destroy the lipid bilayers of the skin. Ethanol and isopropanol as alcohol can be used. Neutral liposomes tend to stick together, and this leads to leakage of the medicinal substance (MS) from the vesicles. However, ethosomes contain ethanol in their composition, which modifies the total charge of the system, which leads to resistance to agglomeration. The increase of ethanol

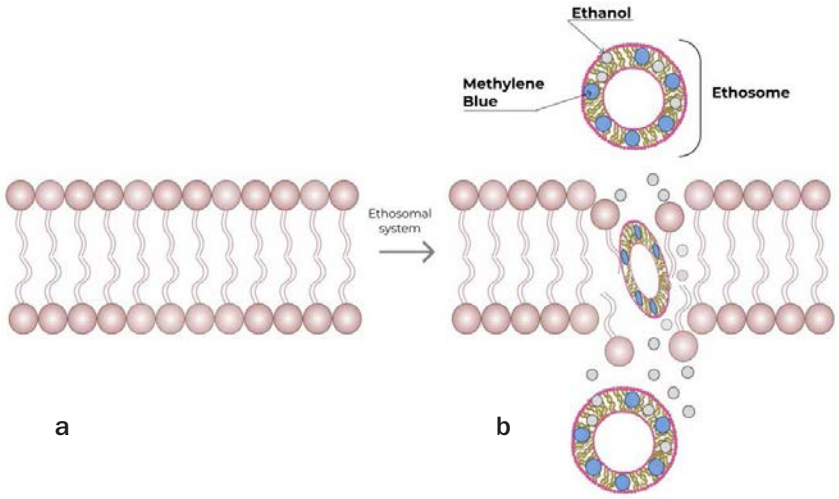


Рис. 2. Предполагаемый механизм проникновения этосомальной системы через мембрану роговой слой кожи:
a – упорядоченные липидные бислои
b – липидный бислой, разрушенный этанолом и накопившиеся мягкие, податливые этосомы
Fig. 2. The proposed mechanism of penetration of the atosomal system through the membranes of the stratum corneum:
a – ordered lipid bilayers
b – lipid bilayer, disturbed by ethanol and accumulated soft, malleable ethosomes

concentration from 15 to 45% leads to membrane fluidity, thereby increasing the efficiency of MS capture. However, a further increase in concentration may lead to a violation of the tightness of the membrane. Glycols act as surfactants (surfactants), they enhance the penetration of vesicles. Propylene glycol or transcutol among glycols are used. Additional stability of vesicles by adding cholesterol (0.1-1%) to the composition can be achieved. Oxidative degradation from light of lipids of ethosomal vesicles can be minimized by using the antioxidant α-tocopherol (a type of vitamin E and has the number E307).

Fig. 2 shows the structural form and the principle of penetration of liposomal vesicles loaded with MB.

Materials and methods

Materials

Ethosomes

Egg phospholipids, propylene glycol (PG), bidistilled water (DDW), ethyl alcohol (EtOH), carbopol were used for the preparation of ethosomal vesicles, and PS methylene blue as a therapeutic substance and a method for identifying the depth of penetration on a confocal microscope was used.

LESA-01 BIOSPEC system

The diagram of the portable system used in Fig. 3 is shown. The signal from the laser or lamp (1) via a U-shaped optical fiber (3) to the tissue under study is transmitted. The distal end of the fiber (5) receives scattered a fluorescent signal to the receiving fibers that surround the exciting central fiber. At the output end of the optical fiber connected to the spectrometer (2), the fibers form a straight line (6). At the entrance to the spectrometer there is a narrow-band light filter that reduces the intensity of the laser signal scattered backwards. The received signal is digitized and displayed on the screen of a PC with integrated UNO (4) software in real time [26]. The diagram of the portable system used in Fig. 3 is shown. The signal from the laser or lamp (1) via a U-shaped optical fiber (3) to the tissue under study is transmitted. The distal end of the fiber (5) receives scattered fluorescent signal to the receiving fibers that surround the exciting, central fiber. At the output end of the optical fiber connected to the spectrometer (2), the fibers form a straight line (6). There is a narrow-band light filter at the entrance to the spectrometer that reduces the intensity of the laser signal scattered backwards. The received signal on the screen of a PC with the built-in UNO (4) software in real time is digitized and displayed [26].

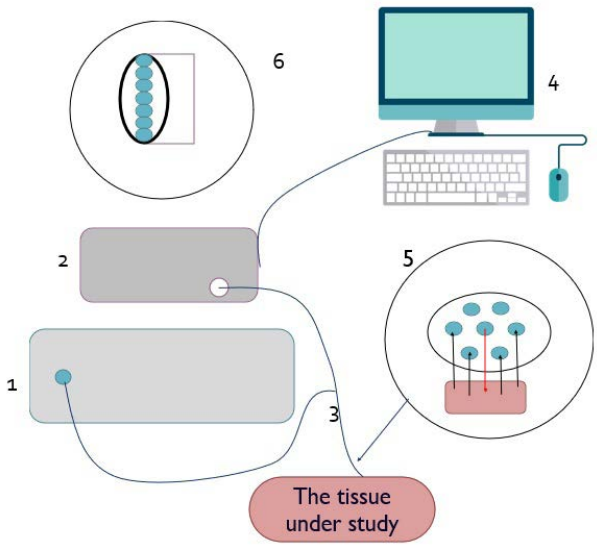


Рис. 3. Схематическое изображение экспериментальной установки, состоящей из: 1 –источника лазерного сигнала, 2 – спектрометр, 3 – оптические волокна, 4 – ПК с программным обеспечением UNO, 5 – торец диагностического катетера, 6 – выход

Fig. 3. Schematic representation of an experimental setup consisting of: 1-laser signal source; 2 – spectrometer; 3 – optical fibers; 4 – PC with UNO software; 5 – working part; 6 – output

Methods

After preparing the formulations for transdermal delivery by cold method, they at the temperature of 3-4°C in the refrigerator were stored. Ethosomal vesicles by the following methods were characterized.

1. Bubble size determination using dynamic light scattering (DLS) and zeta potential using a zeta meter.
2. The content of MS in ethosomal systems can be determined using a spectrophotometer.
3. The study of the drug release kinetics from the ethosomal system in this work when determining the MS formation in vesicles at different ambient temperatures was carried out 4°C, 27°C and 37°C at regular intervals.
4. Using the LESA-01 BIOSPEC spectroanalyzer, studies on human skin and pork skin using a special adapter to determine fluorescence at depth and on the skin surface were carried out.
5. Skin penetration study: the ethosomal preparation ability to penetrate the skin layers was determined using laser confocal scanning microscopy (LCSM).

Results and discussions

Synthesis of ethosomal samples

The classic method of ethosomes synthesis was used. Phospholipid and MB in ethanol were dissolved. Twice distilled water slowly in a thin stream with constant stirring at a speed of 700 rpm using a blender for 5 minutes was added. The ethosomal system at a temperature of 30°C during synthesis to room temperature was kept and then cooled.

Determination of vesicle size and morphology

Fig. 4a shows the results of the size distribution for

samples MB0-MB4. The average size of vesicles dissolved in ethanol, determined by Malvern Zetamaster, was 240.68 nm for the sample CM3.

The size distribution of this ethosomes ranges from tens of nanometers to microns. The size of ethosome depends on can composition of the system. For example, the graph shows an increase in the size of the ethosomes when PG to the samples MS2, MS3 and MS4 is added. Another example may be the formation of a step in the size distribution when carbopol to the composition of MS 4 is added. The smooth surface of the bubbles using SEM was confirmed (Fig. 4b).

Drug release kinetics from ethosomes and penetration depth

The stability of the colloidal solution by Malvern Zetamaster for each sample was determined. Fig. 5 shows the distribution of the zeta potential. According to the data obtained, the ethosomes containing a large amount of ethanol in their composition, which causes a modification of the total charge of the system and gives it a certain degree of steric stabilization, leads to an increase in the stability of the system to agglomeration. According to the graph of the zeta potential of the MS4 sample, there are impurities can be concluded.

All samples underwent studies on the kinetics of MB release at different temperature conditions of 27°C, 37°C and exposure time of 10, 20 and 30 minutes. The optical density of the supernatant on a spectrophotometer was determined. The results of the dynamics of the release of MS from ethosomes under various temperature conditions in the Fig. 6ab.

The exposure time increases, the optical density increases, which indicates the release of MB from vesicles. At the same time, there is an increased release at a temperature of 37°C, which indicates an increase in the release rate when penetrating into the deep layers of the skin.

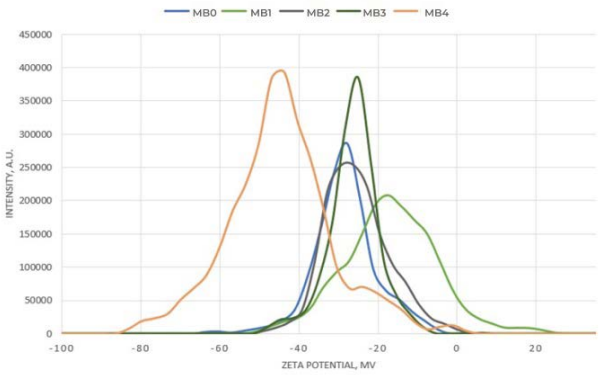
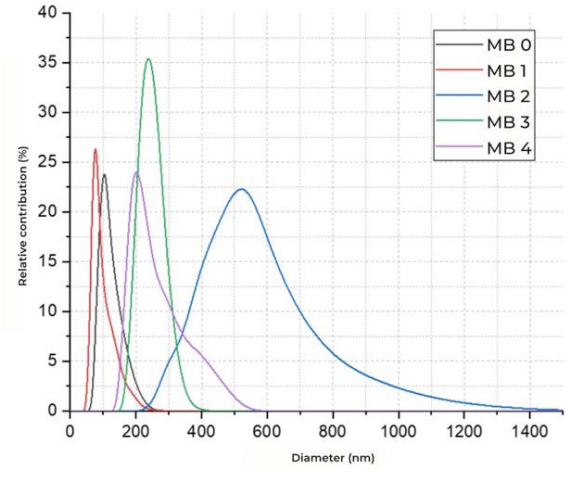


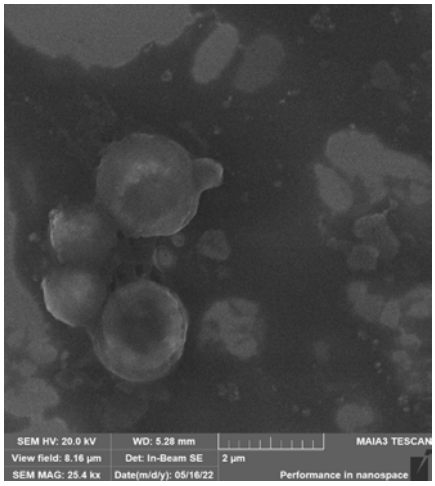
Рис. 5. Распределение дзета-потенциала для образцов MB0-MB4
Fig. 5. Zeta potential distribution for samples MB0-MB4

The kinetics of the release of MB from ethosomes on the patient's skin and pork skin using the LESA-01 BIOSPEC spectrometer were also determined. Obviously, fluorescence wavelength at depth and on the surface of the patient's skin is different, so this method allows you to determine the concentration and depth of MB penetration (Fig. 7a). The fluorescence wavelength of samples (T1, I, II) and pork skin fluorescence after irradiation in the NIR spectral region does not change during measurement (Fig. 7b). This also proves the above-mentioned removal of MB from vesicles at a temperature of 37°C. The depth of penetration of MB into pork skin on a confocal microscope in the area with and without the SC was studied (Fig. 8).

Based on the obtained results, graph 10 was constructed, which shows that a large concentration of ethosomal complexes in the SC is found, and then



a



b

Рис. 4.
a – Распределение по размерам образцов; b – Результаты, полученные после сбора осадка в этаноле с помощью СЭМ
Fig. 4.
a – Distribution of samples by size MB0-MB4; b – SEM results obtained after collecting sediment in ethanol

decreases and a local maximum in the basal layer is observed. There is a sufficient concentration of MB at a depth of more than 1.1 mm, which indicates penetration into the dermis. The decrease rate of the MB concentration is significantly lower in comparison with the generally accepted value manifested by the diffuse mechanism of the drug substance distribution. This means that the use of these ethosomal complexes and their stimulation with light significantly increase the depth of MB penetration.

After characterization of the complexes, it was necessary to determine the effective thickness of the cream layer in order to maintain the constancy of the results obtained. In this study, the LESA-01 BIOSPEC system was used. The above histogram shows the results after applying the M3 sample to the arm with a thickness of 0.3, 0.5 and 0.8 mm. The histogram shows that after irradiation in the NIR region of the spectrum, the intensity value decreases from 0.3 to 0.8 mm. Then, after PDT, the fluorescence intensity on the surface and

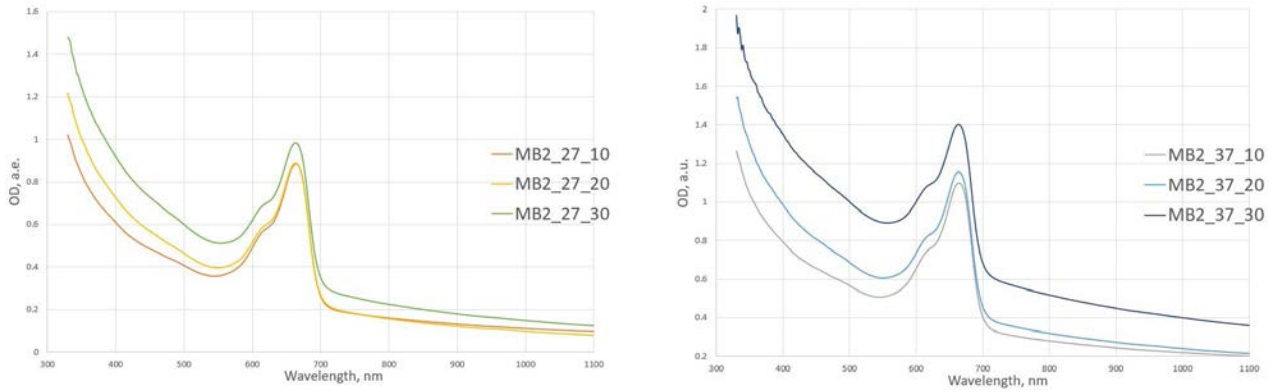


Рис. 6. Зависимость оптической плотности от длины волны для образца МС2 при температуре: a – 27°C; b – 37°C
Fig. 6. Dependence of the optical density on the wavelength for the MB2 sample at an exposure time of 10, 20 and 30 minutes under temperature conditions: a – 27°C; b – 37°C

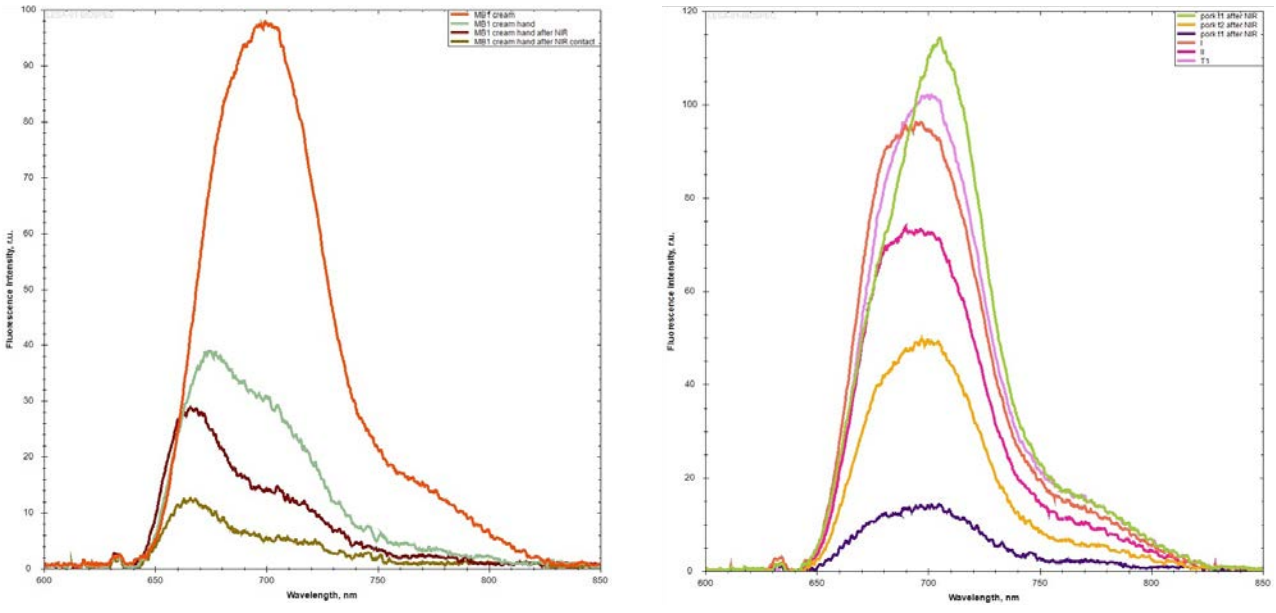
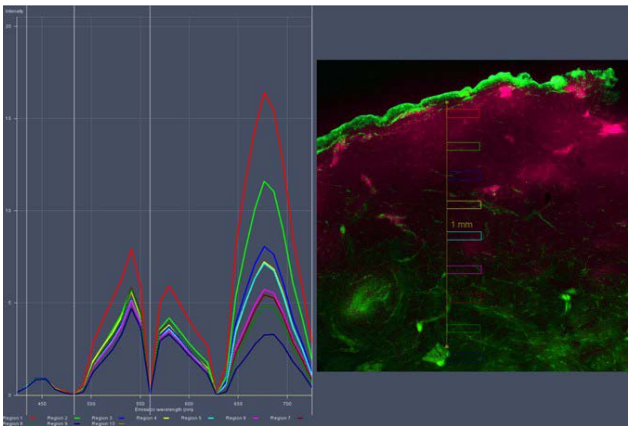
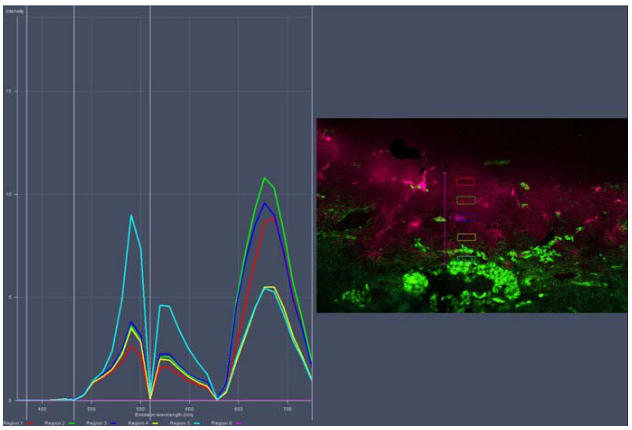


Рис. 7.
a – спектр флуоресценции образца MB1 cream перед нанесением, спектр флуоресценции образца MB1 cream после нанесения на кожу пациента, спектр флуоресценции образца MB1 cream after NIR на поверхности кожи после облучения в БИК области спектра, спектр флуоресценции образца MB 1 cream hand after NIR contact в глубоких слоях кожи до 2 мм после облучения в БИК области спектра; b – T 1, I, II – спектры флуоресценции образцов, pork t1 after NIR, pork t2 after NIR, pork t3 after NIR – спектр флуоресценции образцов на поверхности кожи свиньи после облучения в БИК области спектра
Fig. 7.
a – MB1 cream sample is the sample fluorescence spectrum before application, after application on patient's skin, after irradiation in the NIR spectral region, after NIR contact is the fluorescence spectrum of the sample in the deep layers of the skin up to 2 mm after irradiation in the NIR region of the spectrum; b – T1, I, II – fluorescence spectra of samples, pork t1 after NIR, pork t2 after NIR, pork t3 after NIR – fluorescence spectrum of samples on the surface of pork skin after irradiation in the NIR spectral region



a



b

Рис. 8.
a – результаты, полученные с помощью LCSM, в области с роговым слоем; b – результаты, полученные с помощью LCSM, в области без рогового слоя
Fig. 8.
a – LCSM results in the area with the stratum corneum; b – LCSM results in the area without the stratum corneum

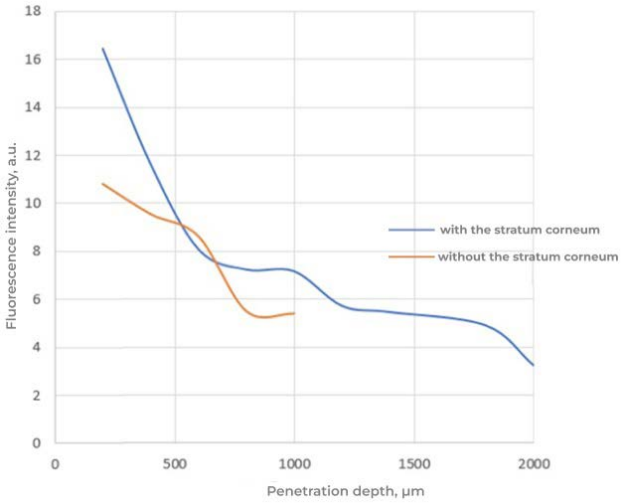


Рис. 9. Зависимость интенсивности флуоресценции от глубины проникновения в роговой слой волны при длине волны 676 нм
Fig. 9. Fluorescence intensity dependence at a wavelength of 676 nm on the penetration depth of the stratum corneum

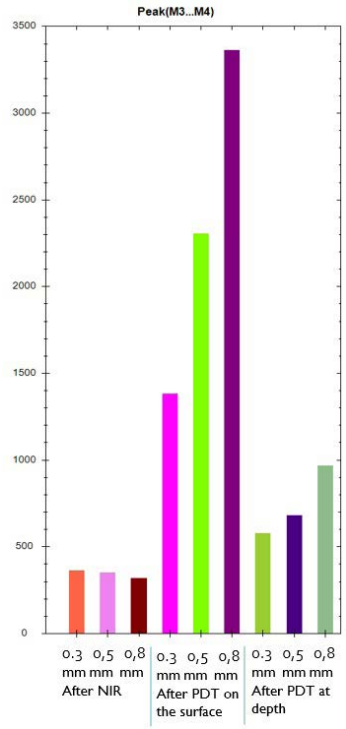


Рис. 10. Гистограмма интенсивности флуоресценции после нанесения крема, облучения в области БИК в течение 20 мин, проведение ФДТ на поверхности и глубине в течение 20 мин
Fig. 10. Fluorescence intensity histogram after applying the cream to the hand and irradiating the NIR for 20 minutes; and conducting PDT for 20 minutes on the surface and at depth

at a depth of up to 2 mm using an optical adapter was assessed. The data after PDT after an increase in laser radiation intensity are presented. According to the data obtained, applying a cream 0.3 mm thick, there is a decrease in accumulation at depth. Therefore, using a cream thickness of 0.3 mm, there is a maximum accumulation at a depth after NIR and maximum photobleaching after PDT.

Conclusion

The research shows that the LESA-01 BIOSPEC system to assess the depth of MB penetration is able, as well as to assess the concentration of accumulated PS in the epidermis and dermis. This system to provide information about the release of PS from vesicles is also able. The effective thickness of the applied preparation was determined. The article presents a comparison of the results of a confocal microscope and the used LESA-01 BIOSPEC system. The LCSM has disadvantages such as labor intensity and the use of animal skin or model samples. The investigation shows the MB introduction from vesicles when working with patient skin and pork skin is different. This is also a plus of working with the LESA-01 BIOSPEC system.

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PHOTODYNAMIC THERAPY TREATMENT OF ORAL CAVITY CANCER IN PATIENTS WITH COMORBIDITIES

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Abstract

We report the experience of radical treatment by photodynamic therapy of patients with squamous cell carcinoma of oral cavity with serious side diseases. Completed treatment of two patients with serious side diseases (HIV infection with associated pulmonary hypertension of high degree and cardiac pathology) suffered from cancer of oral cavity. Extensive surgical treatment and/or aggressive course of chemoradiation therapy were not indicated to them due to concomitant pathology. Both patients were diagnosed with squamous cell carcinoma of oral cavity, with appropriate stage Ist. cT1N0M0. Patients received treatment by photodynamic therapy with chorine photosensitizer in dose 1.0 mg/kg. Options of photodynamic were: output power – 1.5W, power density – 0.31 W/cm^2, light dose – 300 J/cm^2. After one time session of photodynamic therapy, in both cases full response was diagnosed (according to RECIST 1.1). In one case the second session of photodynamic therapy was performed due to concomitant disease of oral cavity – multiply lesions of leukoplakia and after was diagnosed full remission of all lesions. Major adverse event was pain during the first 5-7 days after treatment, curable by painkillers. Follow-up (IQR) was 12 and 18 month respectively with no evidence of progression. It is available to avoid extensive surgical treatment and aggressive course of chemoradiation therapy (as an alternative) with the use of photodynamic therapy. Photodynamic therapy is minimally invasive method of radical treatment of localized squamous cell carcinoma of oral cavity with minimal adverse events, and could be especially relevant in patients with serious concomitant diseases.

Key words: oral cavity cancer, HIV infection, photodynamic therapy, leukoplakia.

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ФОТОДИНАМИЧЕСКАЯ ТЕРАПИЯ ПРИ РАКЕ ПОЛОСТИ РТА У СОМАТИЧЕСКИ ОТЯГОЩЕННЫХ БОЛЬНЫХ

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Резюме

В настоящей работе продемонстрирован опыт радикального лечения соматически отягощенных пациентов с плоскоклеточным раком слизистой оболочки полости рта при помощи фотодинамической терапии. Проведено лечение двух соматически отягощенных пациентов (ВИЧ инфекция с ассоциированной легочной гипертензией высокой степени и выраженной кардиальной патологией), которым

было не показано выполнение обширных хирургических вмешательств и/или проведение агрессивной химиолучевой терапии в связи с наличием выраженной сопутствующей патологии. У обоих пациентов был диагностирован плоскоклеточный рак слизистой оболочки полости рта, распространенность опухолевого процесса соответствовала стадии I cT1N0M0. Пациентам была выполнена фотодинамическая терапия с фотосенсибилизатором хлоринового ряда в дозе 1,0 мг/кг. Параметры облучения: выходная мощность – 1,5 Вт, плотность мощности – 0,31 Вт/см^2, световая доза – 300 Дж/см^2. После одного курса фотодинамической терапии у обоих пациентов диагностирована полная резорбция первичного опухолевого очага (по RECIST 1.1), но в первом клиническом случае был проведен повторный курс фотодинамической терапии в связи с сочетанной патологией слизистой оболочки полости рта – множественными очагами лейкоплакии. В результате лечения так же была отмечена полная регрессия всех очагов лейкоплакии. Основным нежелательным явлением являлась боль в течение первых 5-7 дней после вмешательства, успешно купируемая ненаркотическими анальгетиками. Период наблюдения (IQR) пациентов составил 12 и 18 мес соответственно, без признаков рецидива и метастазов. Благодаря использованию методики фотодинамической терапии у пациентов удалось избежать проведения обширных хирургических вмешательств, а также отказаться от агрессивной схемы химиолучевой терапии, как альтернативы хирургической методике. Фотодинамическая терапия является малоинвазивной методикой радикального лечения локализованного плоскоклеточного рака полости рта с минимальным количеством осложнений, поэтому особенно актуальной эта методика является у пациентов с выраженной сопутствующей патологией.

Ключевые слова: рак полости рта, ВИЧ инфекция, фотодинамическая терапия, лейкоплакия.

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Introduction

According to international and Russian clinical guidelines, the main method of treating oral cancer is the surgical method. In the absence of contraindications, it is recommended to remove the primary lesion within intact tissues [1]. Based on the results of a routine morphological study and evaluation of prognostically unfavorable factors, such as extranodal spread of metastases in the lymph nodes of the neck (ENE +), positive (R1), or close margins (<5mm) of resection, primary tumor with a prevalence of pT3-4, metastases to regional lymph nodes pN2 -3, metastases in the IV and V locoregional groups, the presence of perineural, perivascular, perilymphatic invasion, the method of adjuvant treatment (radiation or chemoradiotherapy) is developed, or dynamic monitoring in their absence is carried out [2,3,4].

An alternative to the surgical technique is external beam radiation therapy (EBRT) or chemoradiotherapy (CRT) alone, with a total focal dose of up to 72 Gy in the area of the primary focus and up to 63 Gy in the area of subclinical distribution [5]. It is possible to use brachytherapy as an independent method of radical treatment of oral cancer using radionuclides Ir-192, CF-252, and others [6,7]. Chemotherapy and targeted therapy are used in combination and/or alone, mainly as a palliative technique in patients who are not indicated for other curative treatments. At the same time, highly toxic schemes with platinum preparations and immunotherapy with pd-L1 inhibitors are recommended in the first line [8,9,10].

Surgery is an invasive method of treatment with the possible development of various complications, up to lethal. At the same time, it is not always possible to conduct full-scale social and cosmetic rehabilitation, which reduces the quality of life. In addition, this type of treatment is not recommended in elderly and/or somatically burdened patients.

CRT, as an alternative to surgical treatment, is most effective with platinum drugs, leading to the development of adverse reactions such as nephrotoxicity, cardiotoxicity, polyneuropathy, hearing loss, and others. With chemoradiation therapy, for the eradication of a tumor of the oral cavity in an independent variant, delivery of the total doses exceeding the tolerance of the surrounding normal tissues is required, which, in turn, leads to the development of such complications as mucositis, osteomyelitis, hyposalivation, long-term non-healing ulcerative processes in the oral cavity and at the site of radiation delivery. Thus, the choice of treatment tactics in elderly and/or somatically burdened patients is a difficult task for an oncologist. Treatment of oral cancer should not only be radical, with a minimum number of complications but should also maintain the patient's quality of life at the "preoperative" level [11].

Photodynamic therapy (PDT) can be used as an independent, radical option for the treatment of malignant neoplasms of the oral cavity, corresponding to stage T1-T2 and with an invasion depth of up to 7 mm, in the absence of alternative methods of radical treatment, such as surgery, EBRT, and CRT. In a retrospective meta-analysis comparing the results of treatment of malignant neoplasms of the oral cavity (surgical method and PDT), the effectiveness was comparable, however, after PDT, there was a significant improvement in the quality of life compared with the surgical method [11].

In the analysis of 43 studies of the effectiveness of PDT in a total of 2121 patients with malignant neoplasms of the head and neck (mainly of the oral cavity), with T1-T2 prevalence, the best response was found in cancer of the

tongue. Complete regression was observed in 94.4% of cases, and 5-year survival was 84.2% [12]. PDT can also be used as a palliative treatment for locally advanced head and neck tumors when other local treatment methods (surgery, radiation therapy) have been exhausted [13,14]. During PDT, not only remote irradiation with laser light can be performed but also interstitial irradiation to reduce the volume of massive tumor foci [15]. In this case, it is possible to achieve remission and/or symptomatic improvement in the form of a decrease in pain, bleeding, and tumor decay. A multicenter study was conducted to evaluate the effectiveness of PDT as a palliative treatment for locally advanced unresectable head and neck cancer, which resulted in a clinical response in 53% of patients. Tumor size decreased by more than 50% in 28% of patients. Complete regression of neoplasms was noted in 17% of cases. The median survival in the study was 226 days, which is longer than after conventional chemotherapy. In addition, there were no significant side effects associated with PDT in patients [16]. The use of PDT does not exclude the possibility of simultaneous or sequential use of other treatment methods, such as surgery, chemoradiotherapy, chemo– and immunotherapy [12,16,17].

Clinical observation 1

Patient P., born in 1978, addressed to the A. Tsyb Medical Radiological Research Centre with complaints of an ulcerative defect in the mucosa of the alveolar bone of the lower jaw on the right (Fig. 1a).

Examination in the retromalar region on the right revealed a tumor with uneven, indistinct edges, ulceration, and bleeding on contact. Moreover, multiple diffuse foci of erosive-ulcerative leukoplakia were noted (Fig. 1b) with the formation of islet foci of stage 2 epitheliitis, moderately painful on contact. Regional lymph nodes were not enlarged.

Histological examination against the background of leukoplakia revealed invasive moderately differentiated squamous cell carcinoma.

Computed tomography revealed a rounded area of increased accumulation of a contrast agent near the angle of the lower jaw. The area was with fuzzy boundaries, with maximum visible dimensions of 10x8 mm, and an invasion depth of up to 4 mm. No destructive changes from the side of the adjacent part of the body of the lower jaw were observed. According to ultrasound data, no enlarged lymph nodes in the neck were found in the supraclavicular and subclavian areas.

The main diagnosis was cancer of the retromolar region on the right of stage I cT1N0M0. During the additional examination, the patient was diagnosed with severe comorbidity – HIV infection of stage 4A (against the background of antiretroviral therapy) and HIV-associated high pulmonary hypertension. Severe cardiac pathology was also diagnosed – chronic heart failure, circulatory disorders 2A, functional class 2 with preservation of the ejection fraction of 56%. Dilated cardiomyopathy was also revealed. The patient had a history of chronic viral hepa-

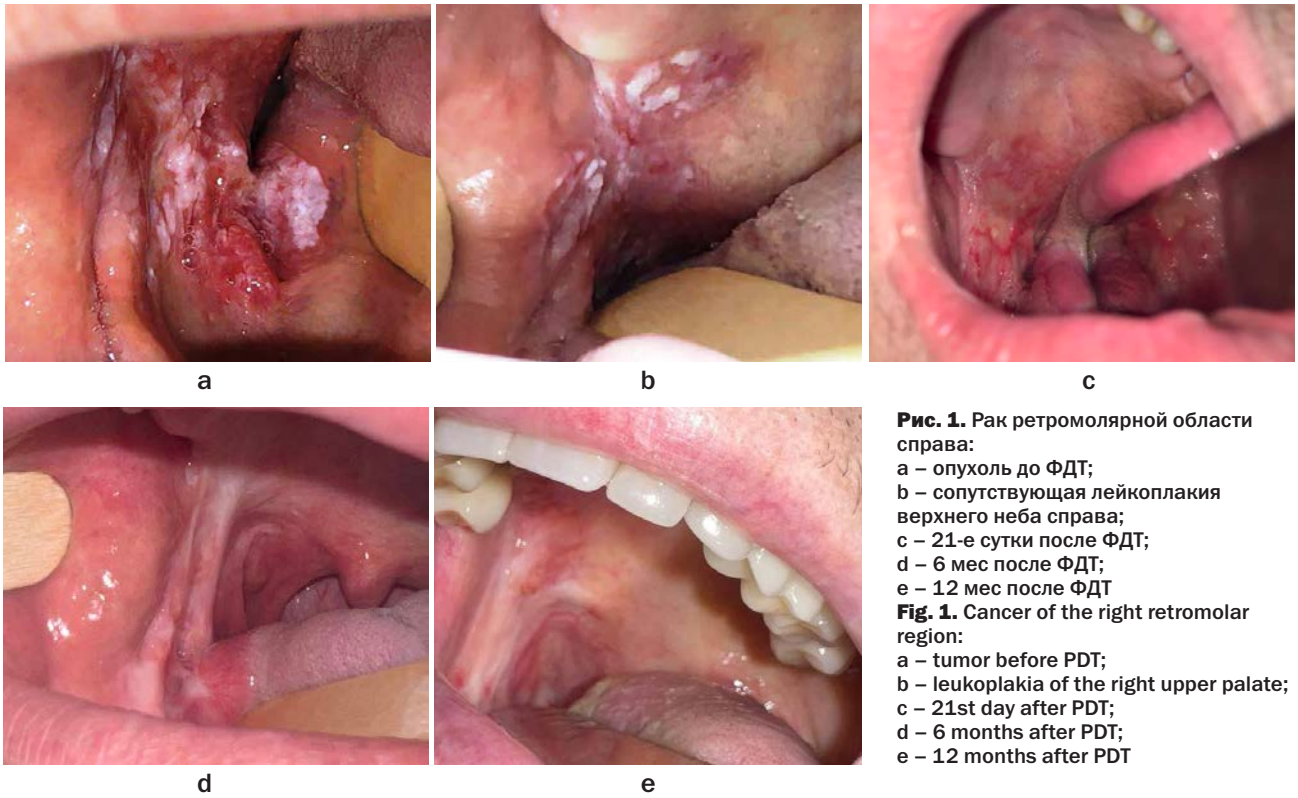

Рис. 1. Рак ретромолярной области справа:
a – опухоль до ФДТ;
b – сопутствующая лейкоплакия верхнего неба справа;
c – 21-е сутки после ФДТ;
d – 6 мес после ФДТ;
e – 12 мес после ФДТ
Fig. 1. Cancer of the right retromolar region:
a – tumor before PDT;
b – leukoplakia of the right upper palate;
c – 21st day after PDT;
d – 6 months after PDT;
e – 12 months after PDT

titis C without replication and chronic obstructive pulmonary disease of bronchitis type. From the endocrinological pathology, primary hypothyroidism in the subcompensation stage, metabolic syndrome, insulin resistance, and obesity of the 2nd degree were diagnosed. A varicose disease of the lower extremities of stage 2 was also present.

Thus, according to the P-POSSUM scale, the risk of lethal complications during surgery reached 40%.

An interdisciplinary consultation was held with the participation of surgeons, radiologists, chemotherapists, and specialists from the PDT department. Taking into account the long-term immunosuppression, multiple foci of leukoplakia, the presence of inflammatory changes in the oral mucosa, and high risks of complications when radical doses of radiation therapy are administered against the background of comorbidities, a decision of conducting an independent PDT course was made.

The patient underwent PDT with the photolon photosensitizer, administered intravenously at a dose of 1.0 mg/kg. For pain management, Ketorolac solution 1.0 ml IM, Promedol 2% 1.0 ml IM, and Relanium 0.5% 2.0 ml IM were used with additionally made local anesthesia with ropivacaine solution. Three hours after the introduction of the photosensitizer, PDT was performed with the laser light source – "Latus 2" (662 nm), remote irradiation of the neoplasm at a power density of 0.31 W/cm^2, the light energy density of 300 J/cm^2, with the number of fields – 1, and the procedure time of 16 min.

After PDT, initial signs of hemorrhagic necrosis, and edema were noted. By the 10th day, a hemorrhagic scab was formed in the form of a fibrin film. By the 14th day, marginal rejection of necrotic tissues, and by the 21st day, active epithelialization (Fig. 1c) occurred.

The patient was discharged from the hospital on the 3rd day after PDT.

Epithelialization of the wound defect occurred on an outpatient basis with drugs with anti-inflammatory and reparative properties. Complete healing with a good functional and cosmetic effect was noted after 8 weeks.

At the follow-up examination 6 months after the treatment, new foci of leukoplakia of small sizes up to 10 mm were diagnosed (Fig. 1d) and a second course of PDT was performed.

Currently (12 months from the start of the treatment), the patient is under dynamic observation without signs of disease progression (Fig. 1f) with a preserved somatic status (ECOG 0).

Clinical observation 2

Patient A., born in 1932, addressed to the A. Tsyb Medical Radiological Research Centre with complaints of a mass in the area of the buccal mucosa on the right.

During a clinical examination of the mucosa of the right cheek in the posterior sections, a tumor of an erosive-ulcerative nature of growth, with fuzzy, uneven edges, up to 1.2 cm in size, was determined (Fig. 2a). Regional lymph nodes were not enlarged.

A moderately differentiated nonkeratinizing squamous cell carcinoma was concluded from the histological examination. To clarify the prevalence of the process, and the presence of regional and distant metastasis, an instrumental examination was performed. According to MRI in the area of the buccal mucosa, a focus of increased accumulation of a contrast agent was identified, with fuzzy boundaries and maximum visible dimensions of 12x8 mm, with an invasion depth of up to 6 mm. Destructive changes from the side of the adjacent part of the body of the lower jaw were not observed. An ultrasound examination of the mucous membrane of the right cheek revealed a hypoechoic formation with a fuzzy, uneven contour, and an invasion depth of 4.7-5 mm. Enlarged lymph nodes in the neck, supraclavicular and infraclavicular areas were not detected.

The main diagnosis was cancer of the buccal mucosa of stage II cT2N0M0. The patient had several comorbidities. From cardiac pathology, the patient suffered from arterial hypertension stage II, risk 4, and coronary heart disease with atherosclerosis of the aorta, heart valves,

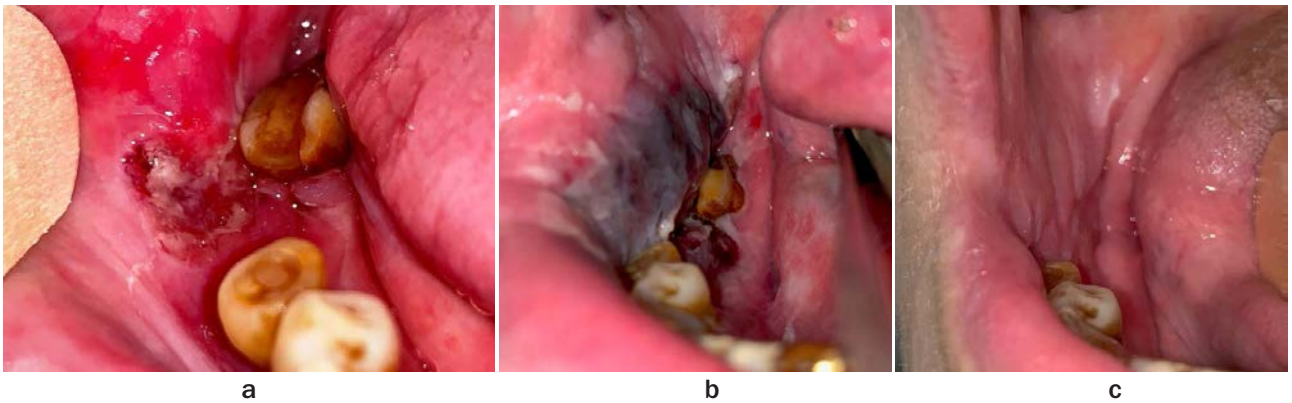

Рис. 2. Рак слизистой правой щеки: a – опухоль до ФДТ; b – 3-е сутки после ФДТ; c – 18 мес после ФДТ
Fig. 2. Cancer of the mucous membrane of the right cheek: a – tumor before PDT; b – 3st day after PDT; c – 18 months after PDT

coronary arteries, circulatory disorders 2A, chronic heart failure, with functional class 2, and preserved ejection fraction of 67%. In history, surgery for an abdominal aortic aneurysm was performed. Besides, chronic kidney disease, stage 4, with a glomerular filtration rate of 28 ml/min, and a varicose disease of the lower extremities 3 tbsp presented.

Taking into account the prevalence of the tumor process, severe comorbidity, age, and extent of the proposed surgical intervention, PDT was proposed as an alternative independent treatment at the inter-departmental consultation.

The patient underwent PDT with photolon administered intravenously at a dose of 1.0 mg/kg. Ketorolac solution 1.0 ml IM, Promedol 2% solution 1.0 ml IM, Relanium 0.5% 2.0 ml IM, and additional local anesthesia with ropivacaine solution were used to stop the pain syndrome.

PDT was carried out three hours after the injection with the laser light source – "Latus 2" (662 nm), remote irradiation of the neoplasm at a power density of 0.31 W/cm^2, the light energy density of 300 J/cm^2, with the number of fields – 1, and the procedure time of 16 min.

The patient was discharged from the hospital on the 3rd day after PDT. The signs of hemorrhagic necrosis and edema were noted locally (Fig. 2b).

At the follow-up period of 18 months, no signs of local recurrence and metastasis were detected (Fig. 2c).

Рис. 3. Сеанс ФДТ
Fig. 3. PDT treatment

Discussion

In the presented clinical examples, PDT was performed on patients from different age groups (43 and 89 years, respectively), in whom, according to the clinical and instrumental examination, the cN0 status of regional lymph nodes was confirmed and the depth of invasion of the primary focus was 4 and 6 mm, respectively. PDT was chosen as an alternative treatment option since surgical intervention or chemoradiotherapy was associated with the risk of developing severe complications due to the presence of severe comorbidities.

PDT was not accompanied by technical difficulties (Fig. 3). In both cases, one irradiation field was sufficient to cover the tumor lesion of the oral mucosa and areas of the potential subclinical lesion (0.5 cm from the visible boundaries of the tumor). In addition, it did not require a long stay in the hospital, the patients were discharged on the 3rd day after treatment.

Adverse events included pain (grade 1 CTCAE) and edema (grade 1 CTCAE), which were managed with nonsteroidal anti-inflammatory drugs and decongestant therapy.

Both patients were diagnosed with complete resorption of the primary tumor focus (according to RECIST 1.1) against the background of a single course of PDT. However, in the first clinical case, a second course of PDT was performed due to a combined pathology of the oral mucosa – multiple foci of leukoplakia. A complete regression of all foci of leukoplakia was noted. The follow-up period (IQR) of patients was 12 and 18 months, respectively, without signs of recurrence and metastases.

Conclusion

The presented clinical experience demonstrates the possibilities of PDT as a minimally invasive, effective method of radical treatment of T1-T2 cancer of the oral mucosa. PDT with chlorin-type photosensitizers may be an alternative treatment option for elderly and somatically burdened patients in whom major surgery and/or aggressive chemoradiotherapy are associated with the development of serious complications. This method is particularly relevant in the treatment of combined pathology of the oral mucosa, such as oncopathology and mucosal leukoplakia. However, for a comprehensive assessment of efficacy and adverse events and the development of indications and contraindications, an analysis of a larger number of patients is required.

In the case of malignant neoplasms of the oral cavity, at certain treatment stages, with both radical and palliative goals, it is possible to use such modern technology as PDT, which has a pronounced antitumor effect and, at the same time, is distinguished by the selectivity of tumor tissue damage, the absence of significant local and systemic side effects and the possibility of repeating courses.

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PREDICTION OF THE EFFECT OF THE PHOTODYNAMIC THERAPY ON SURVIVAL IN PATIENTS WITH STAGE IV OF PANCREATIC CANCER

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Abstract

The article presents the results of a study of survival after complex palliative treatment of patients with malignant tumors of the pancreas stage IV in two comparable groups of patients. The aim of the study is to determine the prognostic factors affecting survival in patients with stage IV pancreatic cancer who received local and systemic photodynamic therapy. In the main group, which consisted of 19 patients with histologically verified stage IV pancreatic maligant tumor, palliative treatment was performed using photodynamic therapy. In the comparison group, consisting of 28 patients with histologically verified malignant tumor of the pancreas stage IV, palliative treatment was performed without the use of photodynamic therapy. On the background of the use of local and systemic photodynamic therapy in the main group it was observed a statistically significant increase in life expectancy compared with the comparison group. The three-month survival of patients who received local and systemic photodynamic therapy is affected by the level of fibrinogen before treatment. The level of fibrinogen above 3.40 g/l makes it possible to predict a decrease in the probability of three-month survival after photodynamic therapy. Thus, complex treatment with the use of photodynamic therapy for stage IV malignant tumors of the pancreas can increase the survival rate of patients.

Key words: malignant tumors of the pancreas, photodynamic therapy, forecasting, survival, prognostic factors.

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ПРОГНОЗИРОВАНИЕ ВЛИЯНИЯ ФОТОДИНАМИЧЕСКОЙ ТЕРАПИИ НА ВЫЖИВАЕМОСТЬ У ПАЦИЕНТОВ С IV СТАДИЕЙ ЗЛОКАЧЕСТВЕННЫХ НОВООБРАЗОВАНИЙ ПОДЖЕЛУДОЧНОЙ ЖЕЛЕЗЫ

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Резюме

В работе представлены результаты исследования выживаемости в двух сопоставимых группах больных после комплексного паллиативного лечения больных со злокачественными новообразованиями поджелудочной железы IV стадии. Целью исследования было определить прогностические факторы, влияющие на выживаемость у больных IV стадией злокачественного новообразования поджелудочной железы, которым планируется проведение локальной и системной фотодинамической терапии. В основной группе, включавшей 19 пациентов с гистологически верифицированным злокачественным новообразованием поджелудочной железы IV стадии, проводили паллиативное лечение с применением фотодинамической терапии. В группе сравнения, включавшей 28 пациентов с гистологически верифицированным злокачественным новообразованием поджелудочной железы IV стадии, проводили паллиативное лечение без применения фотодинамической терапии. На фоне применения локальной и системной фотодинамической терапии

в основной группе наблюдали статистически значимое увеличение продолжительности жизни по сравнению с группой сравнения. Исследования показали, что на трехмесячную выживаемость пациентов, которым планируется проведение локальной и системной фотодинамической терапии, влияет уровень фибриногена до лечения. Уровень фибриногена выше 3,40 г/л позволяет прогнозировать снижение вероятности трехмесячной выживаемости после проведения фотодинамической терапии. Таким образом, комплексное лечение с применением фотодинамической терапии злокачественных новообразований поджелудочной железы IV стадии позволяет увеличить выживаемость пациентов.

Ключевые слова: злокачественные новообразования поджелудочной железы, фотодинамическая терапия, прогнозирование, выживаемость, прогностические факторы.

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Introduction

Pancreatic cancer is an important problem of modern oncology. Being a relatively rare disease and occupying only 3.3% in the structure of cancer incidence in Russia it ranks fifth in the structure of cancer mortality [1, 2, 3, 4, 5].

The modern standard of treatment for patients with malignant neoplasms of the pancreatobiliary zone is complex treatment, in which the surgical method plays the main role. At the same time, due to a long period of slight symptoms and late treatment of patients at the time of diagnosis, more than half of patients already have advanced stage IV of the underlying disease [1, 2]. Most patients can only receive palliative treatment, while the most important factor in successful treatment is the elimination of life-threatening complications such as obstructive jaundice and cholangitis [6, 7, 8, 9, 10]. Against the background of the development of technologies for decompression of the bile ducts, and the emergence of new regimens and drugs for chemotherapy treatment, the prognostic median survival of these patients has not changed over the years, still amounting to 3.7 months for unresectable tumors, which is one of the lowest median survival among tumors of the gastrointestinal tract [2]. Mortality in the first year of life in patients with malignant neoplasms of the pancreas also remains high and amounts to 68% [1].

At the same time, due to the lack of a significant improvement in the results of palliative treatment despite the development of chemotherapy, there is a search for other methods of palliative treatment for this category of patients.

One such method is photodynamic therapy (PDT). PDT is a technique for influencing tumor cells with the help of special drugs that accumulate in them and, becoming chemically active in the presence of light of a certain wavelength and oxygen, lead these cells to die through apoptosis, necrosis, and autophagy. Conducted in 2002 and 2014 studies of the effect of contact PDT on the outcomes of complex treatment of pancreatic cancer suggest that PDT in combination with surgical methods is promising in increasing the life expectancy of patients with malignant neoplasms of the pancreatobiliary zone [8, 9].

The aim of this study is to determine the prognostic factors affecting survival in patients with stage IV pancreatic malignancy who are scheduled for local and systemic PDT.

Materials and Methods

An open non-randomized comparative survivorship study included 47 patients with histologically verified stage IV pancreatic malignancy who underwent complex treatment at the regional hepatological center of the Barnaul City Hospital No. (Barnaul, Russia) from 2017 to 2020. The patients were divided into two groups. The inclusion criteria for the study were age from 18 to 95 years, histologically verified diagnosis of stage IV pancreatic malignancy, and signed informed consent for surgical treatment during hospitalization. The exclusion criteria were mortality in less than 24 hours, HIV infection and infection with viral hepatitis B, C, and D, acute myocardial infarction with cardiogenic shock, and blood cancer. The main group included 19 patients who underwent complex palliative treatment with PDT. The comparison group included 28 patients who underwent complex palliative treatment without PDT. The distribution of patients into groups was carried out without randomization: patients who signed a consent to PDT due to the presence of contraindications to the use of alternative methods of treatment were included in the main group. Patients who refused PDT were included in the comparison group. The design of the study was approved by the Local Ethics Committee of the "Altai State Medical University" of the Ministry of Health of the Russian Federation (extract from protocol No. 11, November 27, 2017). Comparative characteristics of groups by

sex and age are presented in Table 1. No statistically significant differences were found.

Comparative characteristics of groups according to the histological type of neoplasm are presented in Table 2. No statistically significant differences were found.

Palliative surgical treatment included surgical treatment of life-threatening complications, primarily obstructive jaundice: percutaneous transhepatic mono– and bilobar drainage of the bile ducts, stenting of the bile ducts under ultrasound and X-ray control, and bypass biliodigestive anastomoses. Symptomatic conservative treatment included infusion, detoxification, analgesic, hepatoprotective, and antibacterial therapies [5].

All patients of the main group underwent palliative local and systemic PDT using an intravenous photosensitizer photoditazine (LLC "VETA-GRAND", Russia). Photoditazine was injected intravenously, after dissolution in 0.9% natural saline solution, at a dose of 1-1.4

Таблица 1
Сравнительная характеристика больных по возрасту и полу
Table 1
Comparative characteristics of patients by age and sex

Показатель / Index	Основная группа / Main group		Группа сравнения / Comparison group		p
	M±SD		M±SD		
Возраст / Age	62.53±10.74		60.79±9.27		0.568
	абс./ abs.	%	абс./ abs.	%	p
Женский пол / Female	8	42.11	14	50.00	0.815
Мужской пол / Male	11	57.89	14	50.00	0.815

Примечание: p – статистическая значимость различий между основной группой и группой сравнения.
Note: p – statistical significance of differences between the main group and the comparison group.

Таблица 2
Сравнительная характеристика больных по гистологическому типу новообразования
Table 2
Comparative characteristics of patients by hystology of tumor

Гистологический тип/ Histological type	Основная группа/ Main group		Группа сравнения/ Comparison group		p
	абс./ abs.	%	абс./ abs.	%	
Высокодифференцированная аденокарцинома/ High differentiated adenocarcinoma	3	15.79	5	17.86	0.834
Умеренно дифференцированная аденокарцинома/ Moderately differentiated adenocarcinoma	4	21.05	8	28.57	0.811
Низкодифференцированная аденокарцинома/ Low differentiated adenocarcinoma	8	42.11	12	42.86	0.803
Недифференцированная аденокарцинома/ Non-differentiated adenocarcinoma	3	15.79	2	7.14	0.644
Плоскоклеточный рак/ Squamous cell carcinoma	1	5.26	0	0	0.844
Нейроэндокринный рак/ Neuroendocrine cancer	0	0	1	3.57	0.843

Примечание: p – статистическая значимость различий между основной группой и группой сравнения.
Note: p – statistical significance of differences between the main group and the comparison group.

mg/kg of body weight. In the process of administration, intravenous systemic PDT was performed through peripheral access to the cubital vein using an apparatus for intravenous blood irradiation with a monochromatic light source with a power of 0.7 W at a wavelength of 662-665 nm, an exposure dose of light of 1200-1400 J/cm^2, and a radiation power density of 0.22 W/cm^2. After 5 hours from the end of the infusion, local contact PDT was performed by irradiation using a software specialized two-wave laser device with a power of 0.7 W with monochromatic light with a wavelength of 662 nm with an exposure dose of light of 220 J/cm^2 with a radiation power density of 0.22 W/cm^2 through percutaneous transhepatic antegrade access and endoscopically with video esophagoduodenoscopy through retrograde access under endo-ultrasound control [13]. The purpose of local PDT was to achieve cell apoptosis at the periphery of the neoplasm in combination with a complex decrease in the concentration of atypical cells in the systemic circulation using systemic PDT.

Complex treatment with the use of local and systemic PDT was performed by the following algorithm: fluorescence diagnostics on a laser electronic spectrum device "Biospec" (New Surgical Technologies, Russia), local and systemic PDT on a software specialized two-wave apparatus "LAMI-Helios" (JSC "New Surgical Technologies", Russia) according to TS 9444-001-53807582-2010.

Complications of surgical treatment were assessed using the Clavien-Dindo scale [14]. In all patients of the main group, the indicators of hemostasis, proteolysis, and systemic inflammation were analyzed. Determination of the concentration of fibrinogen in plasma according to Clauss (1957) was carried out with a set of reagents from the company "Technology-Standard" (Russia).

To determine the concentration of tissue plasminogen activator (t-PA) in blood serum, the TECHNOZYM t-PA Ag EDTA ELISA kit for enzyme immunoassay was used (Cat. No. TC12007, Technoclone Herstellung von Diagnostika und Arzneimitteln GmbH, Austria). The optical density of the solution in wells at a wavelength of 450 nm was measured using an Elx808 automatic microplate photometer (BioTec Instruments, Inc., USA).

To determine the concentration of tissue plasminogen activator inhibitor-1 (PAI-1) in blood serum, the TECHNOZYM PAI-1 Antigen ELISA kit for enzyme immunoassay was used (Cat. No. TC12075, Technoclone Herstellung von Diagnostika und Arzneimitteln GmbH, Austria). The optical density of the solution in wells at a wavelength of 450 nm was measured using an Elx808 automated microplate photometer (BioTec Instruments, Inc., USA).

To determine the concentration of tissue factor (TF) in blood serum, the IMUBIND Tissue Factor ELISA kit for enzyme immunoassay was used (Cat. No. REF845, BioMedica Diagnostics, USA). The optical density of the solution in the wells at a wavelength of 450 nm was measured using an automatic photometer for microplates Elx808 (BioTec Instruments, Inc., USA).

To determine the concentration of tissue factor pathway inhibitor (TFPI) in blood serum, the Human Tissue Factor Pathway Inhibitor (TFPI) ELISA Kit was used (Cat. No. ET1005-1, Assaypro, USA). The optical density of the solution in the wells at a wavelength of 450 nm was measured using an Elx808 automatic microplate photometer (BioTec Instruments, Inc., USA).

To determine the concentration of tumor necrosis factor-alpha (TNF-α) in blood serum, the Human TNF alpha total Platinum ELISA kit for enzyme immunoassay was used (Cat. No. BMS2034/BMS2034TEN, Bender MedSystems GmbH, Austria). The optical density of the solution in the wells at a wavelength of 450 nm was measured using an Elx808 automatic microplate photometer (BioTec Instruments, Inc., USA).

Statistical analysis was performed using the SigmaPlot 14.0 statistical software package (registration number 775400014). To compare two unrelated groups, the nonparametric Mann-Whitney test was used, since, according to the Shapiro-Wilk test, all the studied parameters, except for gender and age, had a non-normal distribution in the main group and the comparison group. To compare unrelated groups with a normal distribution, Student's parametric test was used, and for relative values – Fisher's z-test. The results for indicators with a non-normal distribution are presented as median (Me), first (Q1) and third (Q3) quartiles, mean (M) and its standard deviation (SD). The results for indicators with a normal distribution are presented as the mean (M) and its standard deviation (SD). The method of Kaplan-Meier curves was used to estimate the overall life expectancy, and a log-rank test was used for a comparative analysis of survival. To determine the predictive ability of indicators, ROC analysis was used to calculate AUC, cut-off points with sensitivity and specificity, likelihood ratio (LR+ and LR-), and predictive value (p). The critical level of significance of the results of the study was taken as $p < 0.05$.

Results and Discussion

There were no postoperative complications after PDT in patients of the main group. One patient of the main group (5.26%) was diagnosed with gallbladder empyema after stenting the bile ducts. In one patient of the comparison group (3.57%), a seroma of the postoperative suture was detected after hepaticojejunostomy, and in one patient of the comparison group (3.57%), a subhepatic hematoma was detected after percutaneous transhepatic multilobar drainage of the bile ducts.

There were no statistically significant differences between the number of postoperative complications

between the main and the comparison groups (p=0.649). All the complications were of grade IIIa according to the Clavien-Dindo classification.

When assessing life expectancy in parallel compared groups (Table 3), the median survival in the main group was statistically significantly higher compared to the comparison group (p = 0.04) (Fig. 1).

Most studies in the field of PDT of malignant neoplasms of the pancreas were carried out in patients without distant metastatic lesions. In a study by Bown et al. [8], Hugget et al. [9], as well as subsequent studies of various photosensitizers using their methods, described in the review by Karimnia et al. [6], the immediate results of using local PDT in patients with local progression of pancreatic cancer were primarily investigated and the exclusion criterion was the presence of stage IV of the disease in patients. Median survival in these studies ranged from 9.5 to 11 months in patients with stages II and III of malignancy. At the same time, the issue of the possibility of improving long-term outcomes in patients with distant metastases of a malignant neoplasm of the pancreas, which make up the majority of newly diagnosed patients, as well as factors affecting the effectiveness of not only local application of PDT but also long-term outcomes of treatment, remains relevant.

Таблица 3
Сравнительный анализ выживаемости больных
Table 3
Comparative analysis of survival of patients

Группа Group	Медиана выживания, дни Median survival, days Me (Q_1; Q_3)	95% доверительный интервал 95% confidence interval	p
Основная Main	148 (287;72)	86.145-209.855	0.04
Сравнения Comparison	68 (188;35)	61.253-74.7477	

Примечание: p – статистическая значимость различий между основной группой и группой сравнения.
Note: p – statistical significance of differences between the main group and the comparison group.

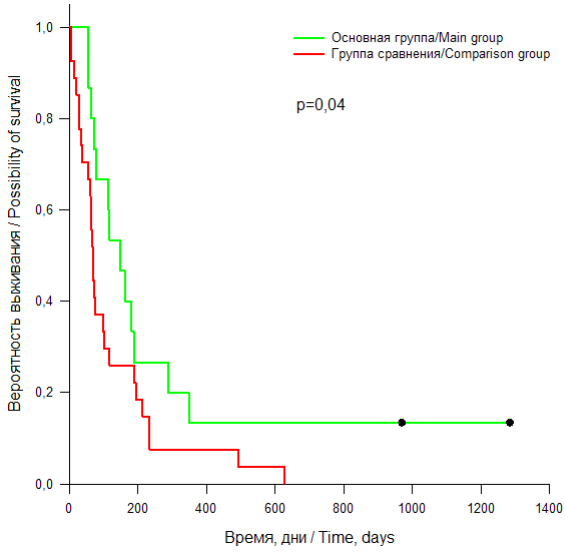

Рис. 1. Сравнительная характеристика выживаемости у пациентов, которым было проведено паллиативное хирургическое лечение злокачественного новообразования поджелудочной железы с применением локальной и системной ФДТ и без ее применения.
Fig. 1. Comparative characteristics of survival in patients who underwent palliative surgical treatment of a malignant pancreatic tumor using local and systemic PDT and without using of it.

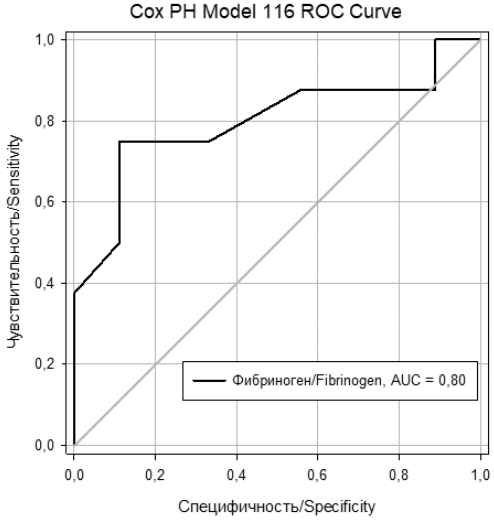

Рис. 2. Результаты ROC-анализа влияния фибриногена на трехмесячную выживаемость у пациентов с раком поджелудочной железы IV стадии.
Fig. 2. Results of ROC analysis of the effect of fibrinogen on three-month survival in patients with stage IV pancreatic cancer.

Таблица 4
Результаты ROC-анализа влияния фибриногена на трехмесячную выживаемость у пациентов с раком поджелудочной железы IV стадии
Table 4
Results of ROC analysis of the effect of fibrinogen on three-month survival in patients with stage IV pancreatic cancer

Показатель Index	AUC (площадь под ROC-кривой) AUC (area under the ROC curve)	Стандартная ошибка (m) Standard error (m)	95% доверительный интервал AUC, (95%CI) 95% confidence interval AUC, (95%CI)	Уровень значимости (p) Significance level (p)
Фибриноген, г/л Fibrinogen, g/l	0,7986	0,1185	0,5664;1,031	0,039
LR+ = 6,75; LR– = 0,28				
TNF-α	0,571429	0,187718	0,2035;0,9394	0,68
TF	0,261905	0,152083	-0,03618;0,5600	0,15
TFPI	0,452381	0,18798	0,08394;0,8208	0,78
TPA	0,571429	0,197777	0,1838;0,9591	0,67
TPA/PAI	0,6	0,185531	0,2364;0,9636	0,57

A causal relationship was analyzed for several markers of hemostasis, systemic inflammation, and proteolysis, taken before PDT, and three-month survival by ROC analysis (Table 4 and Fig. 2). Statistically significant results of the relationship between the level of fibrinogen and three-month survival during PDT were obtained (p=0.039), other indicators had no statistically significant effect (p>0.1). The quality of the model was considered good (the area under the ROC curve was 0.7986). The cut-off limit, at which the sensitivity of the predictive model is 75%, and its specificity is 89%, was 3.40 g/l. The data obtained allow us to conclude that the level of fibrinogen above 3.40 g/l allows us to predict a decrease in the probability of three-month survival after PDT.

Changes in the hemostasis system in cancer patients are one of the leading problems of modern oncology, while thrombotic complications are one of the leading causes of death in cancer patients [16]. Fibrinogen is an important indicator of coagulation hemostasis, abnormalities of which play a leading role in thrombotic complications in malignant neoplasms and affect patient survival [16].

It can be concluded that the results obtained confirm the data obtained in previous studies on PDT. PDT is the method of choice in patients with malignant neoplasms of the pancreas who are not indicated for radical surgical treatment and other methods of palliative treatment due to their high toxicity, which can significantly increase the life expectancy of patients. This is especially true for patients with advanced stage IV of the disease, in whom PDT can significantly increase life expectancy in the absence of side effects from therapy. When choosing a management strategy for such patients, especially those with multimorbidity, the method of prediction of the effectiveness of PDT, which would be available in the routine practice of a surgeon and an oncologist, is important. The method proposed based on the results of the study [17] makes it possible to identify patients with advanced stage IV pancreatic malignancy, in whom PDT will most likely increase life expectancy compared to other methods of palliative treatment.

Conclusion

Thus, complex palliative treatment with the use of PDT of malignant neoplasms of the pancreas allows for increasing the life expectancy of patients without causing side effects on the patient. Based on the foregoing, local and systemic PDT can be recommended as a method of choice in the complex palliative treatment of patients with malignant neoplasms of the pancreas, who are not indicated for radical surgical treatment and other methods of palliative treatment.

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FLUORESCENT DIAGNOSTICS OF NON-MELANOMA SKIN CANCER

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Abstract

Fluorescent diagnostics is a promising method for diagnosing non-melanocytic skin tumors, which makes it possible to identify clinically undetectable skin cancer foci and clarify the margin of the tumor lesion. The main drugs for fluorescent diagnostics are drugs based on 5-aminolevulinic acid and its methyl ester. Sensitivity indicators of fluorescent diagnostics in basal cell, squamous cell carcinoma and extramammary Paget's disease reach 79.0-100.0%, specificity – 55.6-100%. But the effectiveness of this method may be reduced due to hyperkeratinization, keratinization, and the presence of necrotic tissue on the surface of tumor foci. Comparative studies of the results of fluorescent diagnostics and histological mapping during tumor removal using Mohs micrographic surgery showed approximately equal results in the determining of the tumor edges by these methods, which indicates that safe and technically easily performed fluorescent diagnostics can serve as a good alternative to Mohs micrographic surgery, one of the most accurate, but rather labor-intensive and technically complex method for determining the margin of skin cancer foci.

Key words: fluorescent diagnostics, skin cancer, basal cell carcinoma, squamous cell carcinoma, Paget's extramammary desease, tumor margin, 5-aminolevulinic acid, 5-aminolevulinic acid methyl ester.

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ФЛУОРЕСЦЕНТНАЯ ДИАГНОСТИКА ПРИ НЕМЕЛАНОЦИТАРНЫХ ОПУХОЛЯХ КОЖИ

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Резюме

Флуоресцентная диагностика – перспективный метод диагностики немеланоцитарных опухолей кожи, который позволяет выявить клинически не определяемые очаги рака кожи и уточнить границы распространения опухолевого процесса. Основными лекарственным препаратами для проведения флуоресцентной диагностики являются лекарства на основе 5-аминолевулиновой кислоты и ее метилового эфира. Показатели чувствительности флуоресцентной диагностики при базальноклеточном, плоскоклеточном раке кожи и экстрамаммарном раке Педжета достигают 79,0-100,0%, специфичности – 55,6-100%. Эффективность этого метода может снижаться за счет гиперкератинизации, ороговения и присутствия некротической ткани на поверхности опухолевых очагов. Сравнительные исследования результатов флуоресцентной диагностики и гистологического картирования при удалении опухоли методом микрографической хирургии Мооса показали высокую корреляцию результатов определения краев опухоли этими методами, что свидетельствует о том, что безопасная и технически легко выполнимая флуоресцентная диагностика может служить хорошей альтернативой микрографической хирургии Мооса – одному из наиболее точных, но достаточно трудозатратному и технически сложному методу определения границ очагов рака кожи.

Ключевые слова: флуоресцентная диагностика, рак кожи, базальноклеточный рак кожи, плоскоклеточный рак кожи, экстрамаммарный рак Педжета, край опухоли, 5-аминолевулиновая кислота, метиловый эфир 5-аминолевулиновой кислоты.

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Introduction

Tumor lesions of the skin are one of the most common neoplasms in the structure of oncological morbidity. In recent decades, there has been a significant increase in the incidence of skin cancer in the world. In 2021, 68,240 cases of skin cancer (except for melanoma) were detected in Russia, and 442 619 patients diagnosed with skin cancer (except for melanoma) were under dispensary registration by the end of 2021 [1]. Successful treatment of patients with malignant skin tumors is based on the implementation of adequate specialized treatment, which is ensured only by timely and accurate diagnosis with an assessment of the exact margin of the tumor lesion. Diagnosis of tumors and precancerous lesions of the skin is based on the data of the clinical picture obtained during a visual external examination of the patient, and instrumental research methods. To detect skin cancer, fluorescence diagnostics (FD) is successfully used – a study based on the selective accumulation of a photosensitizer or the induction of the formation of endogenous photosensitizers – porphyrins – in the tumor tissue, followed by registration of their fluorescence when irradiated with light of a certain wavelength [2,3].

There are few works devoted to the use of FD as a diagnostic method in clinical practice. A search in the Pubmed database for the keywords "fluorescent diagnostics, photodiagnostics, photodynamic diagnostics, photosensitizer, skin tumors" revealed about 150 scientific papers, 80% of which were devoted to studying the kinetics of photosensitizer accumulation in tumor foci and healthy skin using local fluorescence spectroscopy for optimization methods of photodynamic therapy with various photosensitizers, as well as the study of the phenomenon of autofluorescence of tumor tissues. Only 19 articles over the past 20 years have been relevant to the subject of this review. The data presented in them was analyzed and included in the review.

In most studies, 5-aminolevulinic acid (5-ALA) and its derivatives are used to perform fluorescent diagnosis of non-melanocytic skin tumors. 5-ALA is an intermediate metabolite in heme biosynthesis. Exogenous administration of 5-ALA increases the rate of production of photoactive protoporphyrin IX (PPIX) in all cells of the body in which the process of heme biosynthesis occurs. The enzyme ferrochelatase catalyzes the conversion of PPIX to photoinactive heme. In unaltered cells, this process occurs rather quickly (2–4 h). In tumor cells, due to the higher activity of the enzymes of the initial stage of heme synthesis (in particular, porphobilinogen deaminase), as well as a decrease in the activity in them of ferrochelatase (due to the limited availability of Fe^{2+}), photoactive PPIX accumulates. An increase in the concentration of PPIX in the tumor occurs within a few hours, and its high level is maintained for up to 24 hours, while in normal cells PPIX is quickly converted into photoinactive heme under the action of ferrochelatase. Thus, in the interval from approximately 2 to 24 hours, there is a significant difference (up to 10–15 times) between the concentration of photoactive PPIX in tumor and healthy tissues. This makes it possible to perform the PDT procedure with minimal impact on healthy tissues, to determine the optimal resection margins during surgical removal, and to perform highly efficient fluorescent diagnostics to detect tumors and clarify their margin [2,4,5].

It should be noted that even without exogenous administration of 5-ALA in tissues of locally advanced, especially decaying tumors, the fluorescence of porphyrins is slightly higher than in surrounding healthy tissues due to the biochemical mechanisms described above. C.T. Andrade et al. consider that only additional exogenous administration of 5-ALA and its derivatives in most cases leads to an increase in the fluorescence contrast of tumor and healthy tissues and the possibility of a complete diagnosis [6]. In their studies, these authors demonstrated intense autofluorescence in foci of basal cell carcinoma (BCC), actinic keratosis, and, to a lesser extent, seborrheic keratosis. For actinic keratosis and seborrheic keratosis foci, additional application of topical 5-ALA solution did not lead to an increase in fluorescence intensity. The authors believe that a possible cause was the inefficient penetration of the 5-ALA solution due to hyperkeratinization and necrotic tissue on the surface of the lesions. Also, part of the lesions of BCC and actinic keratosis had extensive keratinization on the surface, which apparently acted as a physical barrier to the penetration of the 5-ALA solution. In such foci, even 60 min after the application of 5-ALA, no increase in fluorescence intensity was noted. A possible solution is to remove the keratin layer by curettage, which is a simple medical procedure. For SC foci, high light absorption by melanin prevents fluorescence visualization both in the case of autofluorescence and in fluorescent diagnostics with exogenous administration of 5-ALA. The fluorescence intensity of BCC foci significantly increased after topical application of a 5-ALA solution, which allowed the authors to recommend FD for the diagnosis of this pathology.

In the study by S. Neus et al. [7] also confirmed the high diagnostic value of FD in patients with BCC. The authors evaluated the effectiveness of FD in patients with foci of BCC on the scalp. Tumor margin were determined using a Wood's lamp by fluorescence after exposure to 20% 5-ALA ointment for 3.5 hours. Tumor resection was performed along the margin determined using FD. 28 foci of BCC were analyzed. In 22 (78.6%) cases, the margin determined by FD completely coincided with the results of histopathological examination. In 6 foci, the margin determined by FD did not correlate with the histopathologically assessed tumor margin. Of these,

4 (14.3%) lesions were not completely removed; Two (7.1%) lesions were completely resected, indicating that the tumor was within the FD-defined margin, but not at the resection margin. Therefore, the rate of incomplete removal of BCC lesions was 14.3% (4/28). FD sensitivity and specificity were 79% and 100%, respectively.

In 2015 E.V. Filonenko [2] published the results of a study on the efficacy of FD with 5-ALA (oral solution at a dose of 1.0 mg/kg 3 hours before diagnosis) in 237 patients with BCC, squamous cell carcinoma (SCC) and metatypical skin cancer. In 100% of patients, FD made it possible to clarify the margin of tumor foci. In 118 patients, 506 foci of additional fluorescence were detected, of which 63 patients were diagnosed with skin cancer during morphological examination. The sensitivity of the method was 100.0%, the specificity was 55.6%

Similar results were obtained in other studies of FD in patients with BCC and SCC. So, in the study of J. Liutkeviciute-Navickiene et al. [8] FD with 5-ALA and 5-ALA methyl ester (MAL) as a topical application was performed in 126 patients with CCCC and SCC. The sensitivity of the method was 95.4%, the specificity was 88.6%. In the work of Y. Won et al. [9] FD with ME-5ALA using computerized analysis of fluorescent images was performed in 10 patients with BCC. The sensitivity of the method was 82.6%, the specificity was 94.1%.

In recent years, the number of studies has been published in which the effectiveness of FD with 5-ALA and its derivatives was evaluated in comparison with Mohs micrographic surgery (MMS), one of the most accurate, but rather labor-intensive and technically complex method for determining the margin of skin cancer foci. When using MMS, the neoplasm is excised with simultaneous histological examination of layered sections. The affected tissue is removed layer by layer, and the removed layers are sent for urgent histological analysis. If malignant cells are found in it, tissue excision continues. This happens until the entire next resected area consists of healthy tissues. MMS provides intraoperative microscopic assessment of 100% of the lesion margins, which allows the removal of only the affected tissue and reduces the recurrence rate [10]. The high prevalence of skin cancer, combined with the costs and time associated with MMS, determines the relevance of developing new methods for refining tumor margins that could increase the effectiveness of MMS or serve as an alternative to MMS [11,12]. These studies have shown a correlation between the results of determining the edges of the tumor by the FD method with the results of determining the edges of the tumor by the MMS method.

In the study by B. Stenquist et al. [13] in 12 patients with basal cell skin cancer (BCC), fluorescence after application of 5-ALA ointment and histological mapping using MMS correlated in 42% of tumors with partial correlation in other lesions. A.M. Wennberg et al. [14] showed a strong correlation of tumor margins on digital fluorescence imaging after pre-exposure of 5-ALA ointment with MMS histological mapping in 50% of patients. Another study [15] evaluated the effectiveness of using FD with MAL to clarify the margin of the tumor lesion of BCC foci. The exposure time of the ointment with a photosensitizer was increased compared to standard methods and averaged 13 hours. The study involved 27 patients with BCC with lesions with an average diameter of 1.05 ± 0.35 cm. The margin of tumor foci was determined using a digital fluorescence imaging system with assessment of PPIX accumulation in tumor tissue relative to normal tissue. The BCC lesions were then surgically removed according to defined margins using MMS. In 12 (44.44%) of 27 foci, the edge of the tumor, according to FD, coincided with the histopathological picture. The average fluorescent contrast value was 2.7. Although 15 pigmented BCC lesions showed reduced or absent fluorescence in the center, fluorescence in their periphery was used as a guide for resection. In these 15 lesions, the number of additional MMS resection steps required to clear the lateral margin, each with an additional 1 mm excision, was one step in 14 (93.3%) BCC lesions and two steps in 1 (6.6%) BCC lesion. Results similar in effectiveness were obtained in a study by Jeon et al. [sixteen]. In 38 patients with SCC, the margin of the tumor was determined by the FD method with 5-ALA (20% ointment) or MAL (16% ointment), after which the tumor was resected. After tumor resection, the mean number of additional MMS resection steps required to completely remove the tumor was 1.37±0.75. In 29 patients who underwent resection without FD (control group), the average number of additional resections was 1.83±0.89 ($p = 0.02$). In the FD group, 29 cases (76.3%) required one additional resection to completely remove the tumor, while nine (23.7%) required two or more resections. In the group without FD, one additional resection was required in 13 cases (44.8%) for complete excision of the tumor, while in 16 cases (55.2%) two or more resections were required ($p = 0.008$).

The literature describes attempts to use the FD method for the differential diagnosis of tumor and precancerous skin diseases with determining the stage of the disease by fluorescence intensity. So, T. Smits et al. [17] demonstrated that fluorescent diagnostics with 5-ALA derivatives cannot be used as a non-invasive diagnostic procedure to differentiate different stages of actinic keratosis, since no significant differences in fluorescence were found between different stages of actinic keratosis. However, fluorescent contrast is generally higher in Bowen's disease than in KIN I and KIN II lesions. The highest fluorescent contrast was found in

squamous cell carcinoma lesions, but the small number of such lesions (3 out of 86 biopsied lesions were classified as SCC) and the fact that they belonged to the same patient prevented a reliable statistical comparison. In further studies [18], the authors showed that only KIN I and KIN III foci show significant differences in fluorescent contrast values (1.3 and 2.5, respectively; $p<0.05$). The highest contrast ratios were determined in the foci of microinvasive SCC – 2.7.

A number of studies have shown the high efficacy of FD in patients with extramammary Paget's disease (EMPD). In the work of M. Wan et al. [19] in 21 patients with EMPD, the margin of the tumor lesion was determined by the FD method (photosensitizer – 20% 5-ALA ointment, exposure time 3.5 h) and the method of multiple exploratory biopsies (MSBs). The authors showed a strong correlation between the margin of the tumor lesion, determined by the FD method, and the margin, determined by the biopsy method with histological examination.

The high diagnostic value of FD in EMPD was also confirmed in the study by M. Wu et al. [20] The study included 36 patients. 5-ALA was used as a photosensitizer in the form of a 20% solution with an exposure time of 2 h. Tumor margin were determined visually, using FD and FD methods in combination with reflective confocal microscopy. 130 samples were taken from patients, in which the tumor process was confirmed. Out of 130 sections with a pathologically confirmed tumor process, 83 sections (63.8%) were located outside the macroscopically defined margin of tumor foci with a distance of 3.5±3.1 mm and a median of 2.7 mm; 46 (35.4%) sections were beyond the borders of the marker line of tumor foci, determined by the FD method with a distance of 2.1±1.7 mm and a median of 1.5 mm; 27 (20.8%) sections – outside the FD marker line with reflective confocal microscopy with a distance of 1.4±1.2 mm and a median of 0.9 mm.

Despite the high sensitivity and specificity of skin cancer FD, the development of modified FD methods aimed at increasing these parameters continues. For example, van der Beek et al. [21] presented the results of a study demonstrating a significant increase in the specificity and sensitivity of FD with 5-ALA in the form of a liposomal spray using the normalized fluorescence method. This approach reduces the contribution of external factors that distort the fluorescence intensity of PPIX, such as reflection due to non-perpendicular light hitting the skin, absorption of radiation by a thicker stratum corneum, or a change in radiation intensity due to a difference in the distance between the skin and the light source. The technique is based on the simultaneous measurement of PPIX-mediated fluorescence and autofluorescence after exposure to pulsed light with a wavelength of 407 nm. The source of autofluorescence is most likely bound collagen. Since the emission spectra of autofluorescence and fluorescence of PPIX are separated, it is technically possible to simultaneously measure autofluorescence in the green region of the spectrum and fluorescence and fluorescence of PPIX in the red region of the spectrum. At the same time, the system calculates the normalized fluorescence of PPIX. As a result, if there is a variable that affects the intensity of both types of fluorescence, it is possible to filter out this "noise" using data on the intensity of autofluorescence. The recorded fluorescence intensity is visualized with a pseudo color overlay, where red indicates the highest fluorescence and blue indicates the lowest fluorescence in the image. The analyzed focus was considered potentially suspicious for the presence of a pathological process when the fluorescence intensity (normalized or not) was exceeded by 33% or more than fluorescence in normal skin. The use of the normalized fluorescence assessment technique made it possible to increase the sensitivity of FD from 39% to 97% and the specificity from 27% to 100%.

FD can be used not only to determine the margin of a tumor lesion, but also as a method for monitoring the effectiveness of treatment. So, in the study by M. Bosseila et al. [22] evaluated the change in the fluorescent contrast of mycosis fungoides in 22 patients using fluorescence spectroscopy with 20% 5-ALA ointment (3-hour exposure). Diagnosis was performed twice, before and after specialized treatment for 12 weeks, including PUVA therapy with 8-methoxypsoralen and narrowband medium wave UVB therapy (311 nm) in combination with subcutaneous injection of IFN-αinterferon into resistant lesions. Studies have shown that the positive dynamics, confirmed by a decrease in the level of malignant CD4+/CD7– T cells, was accompanied by a decrease in the average fluorescent contrast from 2.2 to 1.94 ($p = 0.009$). Based on the data obtained, the authors conclude that in the case of mycosis fungoides, fluorescent diagnostics can be an effective tool for assessing the response of the tumor focus to therapy.

J. de Leeuw et al. [23] suggested using the FD method for screening for tumor and precancerous diseases in people working outdoors and exposed to constant UV radiation. The study involved 93 volunteers. In each patient, two drugs were used as a pro-photosensitizer: 5-ALA and MAL, which made it possible to compare their effectiveness. MAL in the form of a 16% cream was applied under an occlusive dressing on the right side of the forehead for 3 hours. Liposomal spray 5-ALA 0.5% was applied every 5 minutes on the left side of the forehead without occlusion for 2.5 hours, followed by 0 5-hour pause to allow the 5-ALA liposomes to fully absorb into the skin. Immediately afterward, fluorescent images were taken of both sides of the forehead. The fluorescent image of

the right (MAL treated) side of the forehead showed a very high and uniform fluorescence intensity in most of the examined skin areas with little difference between normal and diseased skin. The left side (treated with 5-ALA liposomal spray) in most cases showed low fluorescence of normal skin and moderate but distinct fluorescence of non-melanocytic skin tumor foci. At the same time, the authors note that the significantly lower absorption of 5-ALA in liposomes in normal skin leads to a lower degree of photosensitivity, and the faster clearance of 5-ALA provides a better safety profile compared to MAL cream (8 hours versus 36 hours of photosensitivity). As a result of the study, fluorescence was detected in 287 lesions in 61 patients. According to histological examination in 28 patients, positive fluorescence was detected in 212 benign lesions. Including 22 patients in 204 fluorescent foci, sebaceous hyperplasia was diagnosed, and the remaining 8 foci of false positive fluorescence in 6 patients corresponded to viral warts, benign lichenoid inflammation and dysplastic melanocytic nevi. In 29 patients, actinic ketosis was histologically confirmed in 71 fluorescence foci. 4 patients had 3 foci of BCC and 1 lesion of SCC. False-negative fluorescence was detected in only one lesion located on the scalp (negative fluorescent detection of a lesion clinically suspected and histologically confirmed as actinic keratosis). In 5 patients, 5 foci of actinic keratosis were detected by a fluorescent method and subsequently confirmed by histological examination, previously not noted by either the patients or the doctors who conducted the examination. When the fluorescence detection system used in this study was combined with clinical examination and dermatoscopy, the specificity of this method was 92%.

One paper was found in the literature devoted to evaluating the effectiveness of the use of photosensitizers for FD not based on 5-ALA and its derivatives. So, in the work of S.V. Kamrava et al. [24] FD with a photosensitizer based on chlorin e6 was performed in 40 patients with SCC (i.v. administration at a dose of 1.0 mg/kg 4-6 hours before diagnosis). The sensitivity of the method was 90%, specificity – 80%, accuracy – 87.5%, positive predictive value – 93%, negative predictive value – 72%.

In table the results of the main studies on the effectiveness of FD in nonmelanocytic skin tumors are summarized.

Таблица

Сводные данные результативности применения флуоресцентной диагностики у пациентов с немеланоцитарными опухолями кожи

Table

Summary data on the effectiveness of the use of fluorescence diagnostics in patients with non-melanoma skin cancer

	Авторы Authors	Число пациентов Number of patients	Диагноз Diagnosis	Фотосенсибилизатор Photosensitizer	Длина волны излучения Radiation wavelength	Результаты Results
1	Won и соавт. 2007, [9] Won et al. 2007, [9]	10 пациентов 10 patients	БКРК BCC	МЭ-АЛК 20% мазь MAL 20% ointment	Лампа Вуда, λ макс 365 нм Wood's lamp, λ max 365 nm	Чувствительность 82,6% Специфичность 94,1% Sensitivity 82.6% Specificity 94.1%
2	Smits и соавт. 2007, [17] Smits et al. 2007, [17]	14 пациентов 14 patients	86 очагов, в том числе 3 ПКРК, 67 актинический кератоз (32 KIN I, 18 KIN II, 17 KIN III), 10 нормальная кожа 86 lesions, including 3 SCC, 67 actinic keratosis (32 KIN I, 18 KIN II, 17 KIN III), 10 normal skin	5-АЛК 20% мазь 5-ALA 20% ointment	Ксеноновая лампа с фильтром 370-440 нм Xenon light source with a custom band pass filter 370–440 nm	Не обнаружено существенных различий во флуоресценции между различными стадиями актинического кератоза. Флуоресцентная контрастность при болезни Боуэна, как правило, выше, чем при поражениях KIN I и KIN II No significant differences in fluorescence were found between different stages of actinic keratosis. Fluorescent contrast in Bowen's disease is generally higher than in KIN I and KIN II lesions

3	Neus и соавт. 2008, [7] Neus et al. 2008, [7]	28 пациентов 28 patients	БКРК BCC	5-АЛК 20% мазь 5-ALA 20% ointment	Лампа Вуда, λ макс 365 нм Wood's lamp, λ max 365 nm	Частота полного удаления опухолевого очага БКРК – 85,7%, неполного удаления – 14,3%. Чувствительность – 79% Специфичность – 100% The frequency of complete removal of the tumor focus of BCC is 85.7%, incomplete removal is 14.3%. Sensitivity – 79% Specificity – 100%
4	de Leeuw и соавт. [23] 2009 de Leeuw et al. [23] 2009	93 пациента 93 patients	Скрининг опухолевых и предопухолевых заболеваний у людей, работающих на улице и подвергающихся постоянному воздействию УФ-излучения Screening for neoplastic and preneoplastic diseases in people who work outdoors and are constantly exposed to UV radiation	5-АЛК (0,5% 5-АЛК, инкапсулированная в однослойные липосомы размером 50 нм; спрей 5-АЛК 0,5% каждые 5 мин в течение 2,5 ч на пораженный участок кожи) МЭ-АЛК 16% мазь (экспозиция 3 ч) 5-ALA 0.5% 5-ALA encapsulated in 50 nm sized unilamellar liposomes The 5-ALA 0.5% spray every 5 minutes for 2.5 hours to the involved skin area MAL 16% ointment (exposure 3 hours)	LED, λ макс 450 нм LED, λ max 450 nm	Положительная флуоресценция обнаружена у 61 пациента. Из них у 28 – доброкачественные поражения (в том числе у 22 – гиперплазия сальных желез), у 33 – опухолевая и предопухолевая патология (в том числе у 29 – актинический кератоз, у 3 – БКРК, у 1 – ПКРК) Positive fluorescence was found in 61 patients. Of these, 28 had benign lesions (including 22 had sebaceous gland hyperplasia), 33 had tumor and precancerous pathologies (actinic keratosis in 29, BCC in 3 and SCC in 1 patient)
5	Liutkeviciūte-Navickiene и соавт. 2009, [8] Liutkeviciūte-Navickiene et al. 2009, [8]	126 пациентов 126 patients	ПКРК и БКРК SCC and BCC	5-АЛК и МЭ-АЛК (экспозиция 2-4 ч) 5-ALA and ME-ALA (exposure 2-4 hours)	λ макс 378-426 нм λ max 378-426 nm	Чувствительность 95,4% Специфичность 88,6% Положительная прогностическая ценность 6,1% Отрицательная прогностическая ценность 96,3% В 30% случаев границы опухолевой ткани при применении МЭ-АЛК определялись более четко, чем при применении 5-АЛК Sensitivity 95.4% Specificity 88.6% Positive predictive value 6.1% Negative predictive value 96.3% In 30% of cases, the margin of the tumor tissue were more clearly defined with MAL than with 5-ALA
6	Kleinpenning и соавт. 2010, [18] Kleinpenning et al. 2010, [18]	13 пациентов 13 patients	36 очагов, в том числе 7 ПКРК, 17 актинический кератоз (5 KIN I, 6 KIN II, 6 KIN III), 3 БКРК, 9 нормальная кожа 36 lesions, including 7 SCC, 17 actinic keratosis (5 KIN I, 6 KIN II, 6 KIN III), 3 BCC, 9 normal skin	МЭ-АЛК 16% мазь MAL 16% ointment	Ксеноновая лампа с фильтром 370-440 нм Xenon light source with a custom band pass filter 370–440 nm	Только очаги KIN I и KIN III показывают достоверные различия в значениях флуоресцентной контрастности (1,3 и 2,5, соответственно; $p<0,05$). Самые высокие коэффициенты контрастности были определены у очагов микроинвазивного плоскоклеточного рака – 2,7 Only KIN I and KIN III foci show significant differences in fluorescent contrast values (1.3 and 2.5, respectively; $p<0.05$). The highest contrast ratios were determined in foci of microinvasive squamous cell carcinoma – 2.7

7	Kamrava и соавт. 2012, [24] Kamrava et al. 2012, [24]	40 пациентов 40 patients	ПКРК SCC	Хлорин е6 1 мг/кг за 4-6 ч до ФД Chlorine e6 1 mg/kg 4-6 hours before FD	λ макс 633 нм λ max 633 nm	Чувствительность 90% Специфичность 80% Точность 87,5% Положительная прогностическая ценность 93% Отрицательная прогностическая ценность 72% Sensitivity 90% Specificity 80% Accuracy 87.5% Positive predictive value 93% Negative predictive value 72%
8	Van der Beek и соавт. 2012, [21] Van der Beek et al. 2012, [21]	30 пациентов 30 patients	БКРК Актинический кератоз BCC, actinic keratosis	5-АЛК 5-ALA	λ макс 407 нм λ max 407 nm	Специфичность и чувствительность метода ненормированной флуоресценции существенно ниже, чем у метода обнаружения нормированной флуоресценции (27% и 39% против 100% и 97%) The specificity and sensitivity of the non-normalized fluorescence method is significantly lower than that of the normalized fluorescence detection method (27% and 39% versus 100% and 97%)
9	Jeon и соавт. 2013, [16] Jeon et al. 2013, [16]	38 пациентов в группе ФД и 29 пациентов в контрольной группе 38 patients in the FD group and 29 patients in the control group	ПКРК SCC	19 пациентов: МЭ-АЛК 16% мазь Экспозиция 3 ч 19 пациентов: 5-АЛК 20% мазь Экспозиция 6 ч 19 patients: MAL 16% ointment Exposure 3 hours 19 patients: 5-ALA 20% ointment Exposure 6 hours	Лампа Вуда, λ макс 356 нм Wood's lamp, λ max 365 nm	После резекции опухоли среднее количество дополнительных резекций по Моосу, понадобившихся для полного удаления опухоли, было ниже в группе ФД (1,37±0,75), чем в группе без ФД (1,83±0,89) After tumor resection, the mean number of additional Mohs resections required to completely remove the tumor was lower in the FD group (1.37±0.75) than in the non-FD group (1.83±0.89)
10	Andrade и соавт. 2014, [6] Andrade et al. 2014, [6]	43 пациента 43 patients	71 lesions, including 29 BCC, 31 actinic keratosis, 11 seborrheic keratosis	5-АЛК 5% раствор 5-АЛК был использован для 54 очагов (21 БКРК, 22 актинический кератоз, 11 себорейный кератоз). 10% раствор – для 17 очагов (8 БКРК и 9 актинический кератоз) 5-ALA 5% 5-ALA solution was applied on 54 lesions (21 BCCs, 22 AK, and 11 SK). 10% ALA solution was applied on 17 lesions (8 BCCs and 9 AK)	LED, λ макс 400 нм, 50 мВт/см^2 LED, λ max 400 nm, 50 mW/cm^2	В очагах БКРК отмечено достоверное увеличение интенсивности флуоресценции в 3 раза через 1 час после нанесения раствора 5-АЛК. В очагах актинического и себорейного кератоза интенсивность флуоресценции в течение 1 ч после нанесения раствора 5-АЛК оставалась на уровне аутофлуоресценции In the foci of BCC, a significant increase in fluorescence intensity by 3 times was noted 1 hour after the application of the 5-ALA solution. In the foci of actinic and seborrheic keratosis, the fluorescence intensity remained at the autofluorescence level for 1 hour after application of the 5-ALA solution
11	Filonenko 2015, [2] Filonenko 2015, [2]	227 пациентов 227 patients	БКРК, ПКРК, метатипичный рак кожи BCC, SCC, metatypical skin cancer	5-АЛК (раствор для приема внутрь 1 мг/кг за 3 ч до ФД) 5-ALA (oral solution 1 mg/kg 3 hours before FD)	LED, λ макс 400-405 нм, LED, λ max 400-405 nm	Чувствительность 100,0% Специфичность 55,6% Sensitivity 100.0% Specificity 55.6%

12	El Hoshy и соавт. 2016, [15] El Hoshy et al. 2016, [15]	27 пациентов 27 patients	БКРК BCC	5-АЛК 20% мазь 5-ALA 20% ointment	Ксеноновая лампа с фильтром 370-440 нм Xenon light source with a custom band pass filter 370-440 nm	В 12 (44,44%) очагах границы опухоли, определенные по ФД, полностью совпали с границами, определенными гистопатологически. В 14 очагах гистопатологически границы опухоли были больше на 1 мм, чем определяемые по ФД, в 1 – больше на 2 мм In 12 (44.44%) foci, the margin of the tumor, determined by FD, completely coincided with the margin determined histopathologically. In 14 foci, histopathologically, the margin of the tumor were 1 mm larger than those determined by FD, and in 1 foci, they were 2 mm larger
13	Wan и соавт. 2018, [19] Wan et al. 2018, [19]	21 пациент 21 patients	ЭМРП EMPD	5-АЛК 20% мазь 5-ALA 20% ointment	Лампа Вуда, λ макс 365 нм Wood's lamp, λ max 365 nm	Показана сильная корреляцию между границами опухолевого поражения, определенными методом флуоресцентной диагностики, и границами, определенными методом биопсии с гистопатологией A strong correlation was shown between the margin of the tumor lesion, determined by the method of fluorescence diagnostics, and the margin, determined by the method of biopsy with histopathology
14	Wu и соавт. 2021, [20] Wu et al. 2021, [20]	36 пациентов 36 patients	ЭМРП EMPD	5-АЛК 20% раствор 5-ALA 20% solution	Лампа Вуда, λ макс 365 нм Wood's lamp, λ max 365 nm	Визуальный осмотр – 63,8% ложноотрицательных результатов, ФД – 35,4% ложноотрицательных результатов, ФД + конфокальная микроскопия – 20,8% ложноотрицательных результатов Visual examination – 63.8% of false negative results, FD – 35.4% of false negative results, FD + confocal microscopy – 20.8% of false negative results

*ФД – флуоресцентная диагностика, 5-АЛК – 5-аминолевулиновая кислота, МЭ-АЛК – метиловый эфир 5-аминолевулиновой кислоты, БКРК – базальноклеточный рак кожи, ПКРК – плоскоклеточный рак кожи, ЭМРП – экстрамаммарный рак Педжета
*FD – fluorescent diagnostics, 5-ALA – 5-aminolevulinic acid, MAL – 5-aminolevulinic acid methyl ester, BCC – basal cell carcinoma, SCC – squamous cell carcinoma, EMPD – extramammary Paget's desease

Conclusion

The main indications for FD with 5-ALA are the identification of clinically poorly expressed skin tumors, the search for hidden foci of precancer and skin cancer, as well as the clarification of the margin of the tumor lesion and monitoring the effectiveness of various specialized treatment options.

PPIX-induced fluorescence during preoperative planning is a valuable method for determining the peripheral margin of the tumor. The edges of tumors determined by histological mapping during tumor removal by Mohs micrographic surgery correlate well with the margin detected by tumor-specific fluorescence, which indicates the possibility of using fluorescence diagnostics as a full-fledged alternative to Mohs micrographic surgery – one of the most accurate, but rather labor-intensive and technically complex method determination of the margin of skin cancer foci.

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