ISSN 2413-9432

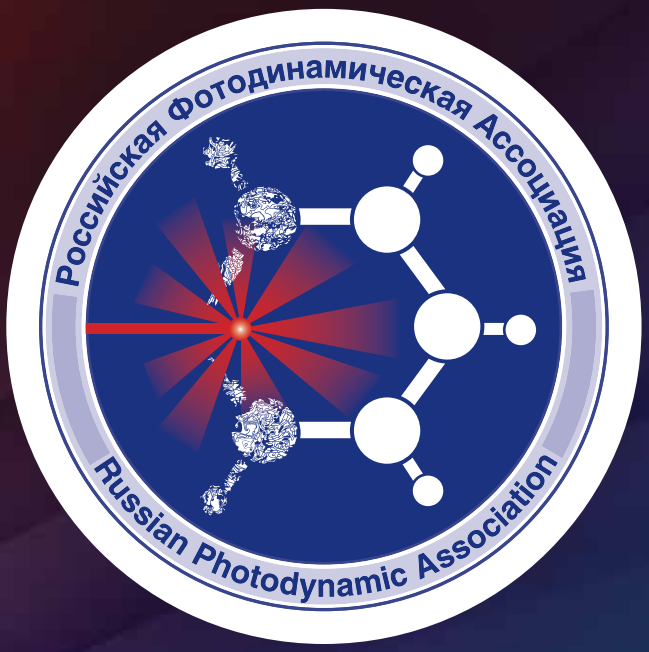
BIOMEDICAL PHOTONICS

Volume 11, # 3, 2022

In the issue:

BMP

Российская Фотодинамическая Ассоциация



www.pdt-association.com

BIOMEDICAL PHOTONICS

BIOMEDICAL PHOTONICS –
research and practice, peer-reviewed, multidisciplinary journal.
The journal is issued 4 times per year.
The circulation – 1000 copies., on a quarterly basis.

The journal is included into the List of peer-reviewed science press of the State Commission for Academic Degrees and Titles of Russian Federation
The journal is indexed in the international abstract and citation database – Scopus.

The publisher «Agentstvo MORE».
Moscow, Khokhlovskiy lane., 9

Editorial staff:
Chief of the editorial staff Ivanova-Radkevich V.I.
Science editor professor Mamontov A.S.

Literary editor	Moiseeva R.N.
Translators	Urlova A.N.
	Romanishkin I.D.
Computer design	Kreneva E.I.
Desktop publishing	Shalimova N.M.

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

Corresponding to:
125284, Moscow, p/o box 13

Registration certificate ПИ № ФС 77–51995, issued on 29.11.2012 by the Federal Service for Supervision of Communications, Information Technology, and Mass Media of Russia

The subscription index of «Rospechat» agency – 70249

BIOMEDICAL PHOTONICS

BIOMEDICAL PHOTONICS –
научно-практический, рецензируемый,
мультидисциплинарный журнал.
Выходит 4 раза в год.
Тираж – 1000 экз., ежеквартально.

Входит в Перечень ведущих рецензируемых научных журналов ВАК РФ.
Индексируется в международной реферативной базе данных Scopus.

Издательство «Агентство МОРЕ».
Москва, Хохловский пер., д. 9

Редакция:

Зав. редакцией	Иванова-Радкевич В.И.
Научный редактор	проф. Мамонтов А.С.
Литературный редактор	Моисеева Р.Н.
Переводчики	Урлова А.Н.
	Романишкин И.Д.
Компьютерный дизайн	Кренева Е.И.
Компьютерная верстка	Шалимова Н.М.

Адрес редакции:
Россия, Москва, 2-й Боткинский пр., д. 3
Тел. 8 (495) 945–86–60
www: PDT-journal.com
E-mail: PDT-journal@mail.ru

Адрес для корреспонденции:
125284, Москва, а/я 13

Свидетельство о регистрации ПИ
№ ФС 77–51995, выдано 29.11.2012 г.
Федеральной службой по надзору в сфере связи, информационных технологий и массовых коммуникаций (Роскомнадзор)

Индекс по каталогу агентства «Роспечать» – 70249

ORIGINAL ARTICLES

ОРИГИНАЛЬНЫЕ СТАТЬИ

REVIEWS OF LITERATURE

ОБЗОРЫ ЛИТЕРАТУРЫ

CASE REPORTS

КЛИНИЧЕСКИЕ НАБЛЮДЕНИЯ

ORIGINAL ARTICLES

CORRELATION OF SPECTROSCOPIC AND STRUCTURAL PROPERTIES OF INDOCYANINE GREEN J-AGGREGATES

Farrakhova D.S.[1], Romanishkin I.D.[1], Yakovlev D.V.[1,2], Maklygina Yu.S.[1], Oleinikov V.A.[2], Fedotov P.V.[1,3], Kravchik M.V.[4], Bezdetnaya L.[5,6], Loschenov V.B.[1]
[1]Prokhorov General Physics Institute of the Russian Academy of Science, Moscow, Russia
[2]Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Science, Moscow, Russia
[3]Moscow Institute of Physics and Technology, Dolgoprudny, Russia
[4]Research Institute of Eye Diseases, Moscow, Russia
[5]Institut de Cancèrologie de Lorraine, Vandoeuvre-lès-Nancy, France
[6]Centre de Recherche en Automatique de Nancy, CNRS, Universitè de Lorraine, Nancy, France

Abstract

Indocyanine green (ICG), when in free form in a liquid, can form stable nanoparticle structures or colloidal solution, while changing its spectroscopic properties. In the work, the aggregation degree and the average size of nanoparticles depending on the concentration of a colloidal solution of indocyanine green (ICG NPs) in the form of J-aggregates were investigated by various methods based on light scattering. The size of nanoparticles is an important parameter from the point of view of clinical application, because the technique of intravenous administration of drugs, in order to avoid microvascular thrombosis and embolism, provides dosage forms with inclusions of individual molecules or their clusters, not exceeding 500 nm diameter. In turn, small nanoparticles less than 30 nm lead to prolonged circulation of the drug in the body with an increased possibility of permeation into cells of healthy tissue. In the course of studies, it was found that an increase in the concentration of ICG NPs in the solution leads to an increase in the average size of spontaneously formed J-aggregates, which, in turn, leads to a decrease in the absorption coefficient in the aggregates. Presumably, this phenomenon, i.e. the established nonlinear dependence of the J-aggregate absorption on its size, can be explained by the formation of absorption centers on the J-aggregate surface in the form of mobile surface molecules. The threshold range of ICG molecule concentration was determined, at which there is a transition from aggregation with an increase in size with a slow addition of ICG J-aggregate molecules in height to a rapid addition in width.

Key words: Mie scattering, dynamic light scattering, indocyanine green, colloidal solution, J-aggregates, scattering indicatrix, aggregation degree.

For citations: Farrakhova D.S., Romanishkin I.D., Yakovlev D.V., Maklygina Yu.S., Oleinikov V.A., Fedotov P.V., Kravchik M.V., Bezdetnaya L., Loschenov V.B. Correlation of spectroscopic and structural properties of indocyanine green J-aggregates, *Biomedical Photonics*, 2022, vol. 11, no. 3, pp. 4–16. doi: 10.24931/2413–9432–2022–11-3-4–16.

Contacts: Farrakhova D.S., e-mail: farrakhova.dina@mail.ru

ВЗАИМОСВЯЗЬ СПЕКТРОСКОПИЧЕСКИХ И СТРУКТУРНЫХ СВОЙСТВ J-АГРЕГАТОВ ИНДОЦИАНИНА ЗЕЛЕНОГО

Д.С. Фаррахова[1], И.Д. Романишкин[1], Д.В. Яковлев[1,2], Ю.С. Маклыгина[1], В.А. Олейников[2], П.В. Федотов[1,3], М.В. Кравчик[4], Л. Бездетная[5,6], В.Б. Лощенов[1]
[1]Институт общей физики им. А.М. Прохорова Российской академии наук, Москва, Россия
[2]Институт биоорганической химии им. академиков М.М. Шемякина и Ю.А. Овчинникова Российской академии наук, Москва, Россия
[3]Московский физико-технический институт, Долгопрудный, Россия
[4]Научно-исследовательский институт глазных болезней им. М.М. Краснова, Москва, Россия
[5]Институт рака Лотарингии, Вандевр-ле-Нанси, Франция
[6]Центр автоматических исследований в Нанси, CNRS, Университет Лотарингии, Нанси, Франция

Резюме

Индоцианин зеленый (ICG), находясь в растворе, способен образовывать стабильные структуры наночастиц или коллоидный раствор, изменяя при этом свои спектроскопические свойства. В работе различными методами, основанными на светорассеянии, были исследованы степень агрегации и средний размер наночастиц в зависимости от концентрации коллоидного раствора наночастиц индоцианина зеленого (ICG NPs) в форме J-агрегатов. Размер наночастиц представляет собой важный параметр с точки зрения клинического применения, так как техника внутривенного введения препаратов, с целью избежания тромбозов микрососудов и эмболии, предусматривает лекарственные формы с включениями, в виде отдельных молекул или их кластеров, не превышающими в диаметре 500 нм. С другой стороны, наночастицы размером менее 30 нм длительно циркулируют в организме и могут проникать в клетки здоровой ткани. В ходе исследований, было установлено, что увеличение концентрации ICG NPs в растворе ведет к увеличению среднего размера спонтанно формируемых J-агрегатов, что в свою очередь ведет к уменьшению коэффициента поглощения в агрегатах. Предположительно, нелинейная зависимость поглощения J-агрегата от его размера, может быть объяснен формированием центров поглощения на поверхности J-агрегата в виде подвижных поверхностных молекул. Был определен пороговый диапазон концентрации молекул ICG, при котором происходит переход от агрегации с увеличением размера с медленным прибавлением молекул J-агрегата ICG в высоту, но с быстрым прибавлением в ширину.

Ключевые слова: рассеяние Ми, динамическое рассеяние света, индоцианин зеленый, коллоидный раствор, J-агрегаты, индикатриса рассеяния, степень агрегации.

Для цитирования: Фаррахова Д.С., Романишкин И.Д., Яковлев Д.В., Маклыгина Ю.С., Олейников В.А., Федотов П.В., Кравчик М.В., Бездетная Л., Лощенов В.Б. Взаимосвязь спектроскопических и структурных свойств j-агрегатов индоцианина зеленого // Biomedical Photonics. – 2022. – Т. 11, № 3. – С. 4–16. doi: 10.24931/2413–9432–2022–11-3-4–16.

Контакты: Фаррахова Д.С., e-mail: farrakhova.dina@mail.ru

Introduction

The fluorescent dye ICG is the only drug with an absorption peak in the near infrared range (NIR), approved for clinical application in most countries of the world [1]. The ICG aqueous solution consists of monomers and H-type dimers with two absorption peaks at 780 and 715 nm, respectively (Fig.1a). ICG is able to form J-aggregates under certain temperature conditions, representing a stable colloid with aggregated nanoparticles (ICG NPs) at high concentrations [2-4]. ICG J- aggregates have an absorption and fluorescence peaks in the NIR range (0,75–1,4 μm) within the biological transparency window [5], which is promising for fluorescent diagnostics [1, 6]. The formation of J-aggregates occurs due to hydrophobic, non-covalent π-π interactions between ICG molecules [3]. In this case, the dipole moments of the electronic transitions of individual molecules are practically aligned parallel to the line connecting their centers through the «head to tail» arrangement (Fig.1b) [3].

The J-aggregates in the ICG NPs colloidal solution have strong changes in spectroscopic properties, such as a narrow J-band in the absorption spectrum and its sharp shift of about 100 nm into NIR. Also, the fluorescence maximum of J-aggregates in a ICG NPs colloidal solution coincides with their absorption peak and demonstrates a behavior close to resonance, at which point, it has a small Stokes shift and insensitivity to the environment [7]. Our studies of ICG NPs colloidal solution have shown their promise for diagnostics of malignant neoplasms, as they increase the circulation of ICG monomers and

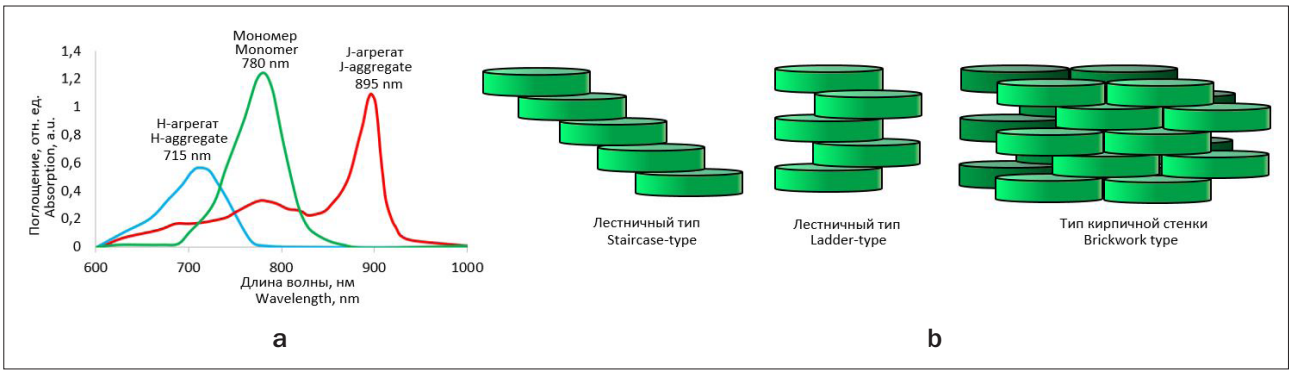


Рис. 1.
a – спектры поглощения ICG;
b – схематическая иллюстрация образования структур J-агрегатов.
Fig. 1.
a – absorption spectra of ICG;
b – schematic illustration of J-aggregate formation.

H-aggregates from 30 min to 2 days [8, 9]. Moreover, when ICG NPs interact with the environment of plasma proteins after intravenous injection, the nanoparticles demonstrate their stability, which can improve fluorescence diagnostics by increasing the circulation of the ICG monomer in the blood system, which makes it possible to predict the pathways of metastasis [10]. Based on experimental data, the determination of the optical characteristic changes of the ICG NPs colloidal solution in the tumor microenvironment remains relevant. In study [11], it was suggested that the nonlinearity of optical characteristics arises due to a decrease in the specific surface of aggregates. However, no assumption was made about the geometric alignment of the J-aggregate nanostructures and the presence of changes (inflection points) in the graphs of the absorption dependence on the concentration.

Materials and methods

The molecular form of ICG, consisting of a diluted polycrystalline powder Pulsion® (Pulsion Medical Systems, Germany), was heated in water to 65°C, and then kept at this temperature for 20 hours to form ICG NPs in accordance with the previously published method [12]. After the formation of J-aggregates, the solution was filtered through syringe filters with 0.40 μm diameter pores to remove large aggregates. A sample with 0.25 μM concentration was selected to study the spectroscopic properties of the ICG NPs colloidal solution. Polylatex calibration spheres with 110 nm diameter were used as a reference sample.

Fluorescence excitation spectra and fluorescence spectral maps were obtained via the Jobin-Yvon NanoLog-4 system (Horiba, Japan). An InGaAs charge-coupled device with 800-1600 nm operating optical range was used as a detector. A xenon lamp (operating range 300-900 nm) with a dual monochromator and 2 nm spectral resolution was used as an excitation source.

Resonant fluorescence registration experiments of ICG NPs colloidal solution were carried out by excitation of the different wavelengths in the 880-915 nm range via Chameleon Ultra II titanium-sapphire laser (Coherent, USA) with 80 MHz frequency and a 140 fs pulse length. For these studies, an installation with a spectrometer and continuous-wave laser was assembled (Fig. 2).

Laser radiation in the 880-915 nm wavelength range with a 1 nm step, passes through a focon into fiber-optic bundle, then through a vertically oriented linear polarizer onto the sample. The scattered laser radiation from the sample passes at 90° angle through a horizontally oriented linear polarizer and an optical fiber into a spectrometer. The crossed polarizer configuration makes possible to exclude the direct laser radiation. 880/10 nm narrow-band filter was also installed in front of the sample to suppress laser radiation (Fig. 2).

For obtaining the scattering indicatrix of the ICG NPs colloidal solution, an installation was assembled on a rotating platform with a receiving optical fiber and fixed optical bundle for delivering laser radiation (Fig. 3). 920 nm wavelength was chosen as laser radiation, for the reason that ICG NPs has no absorbing properties at the wavelength.

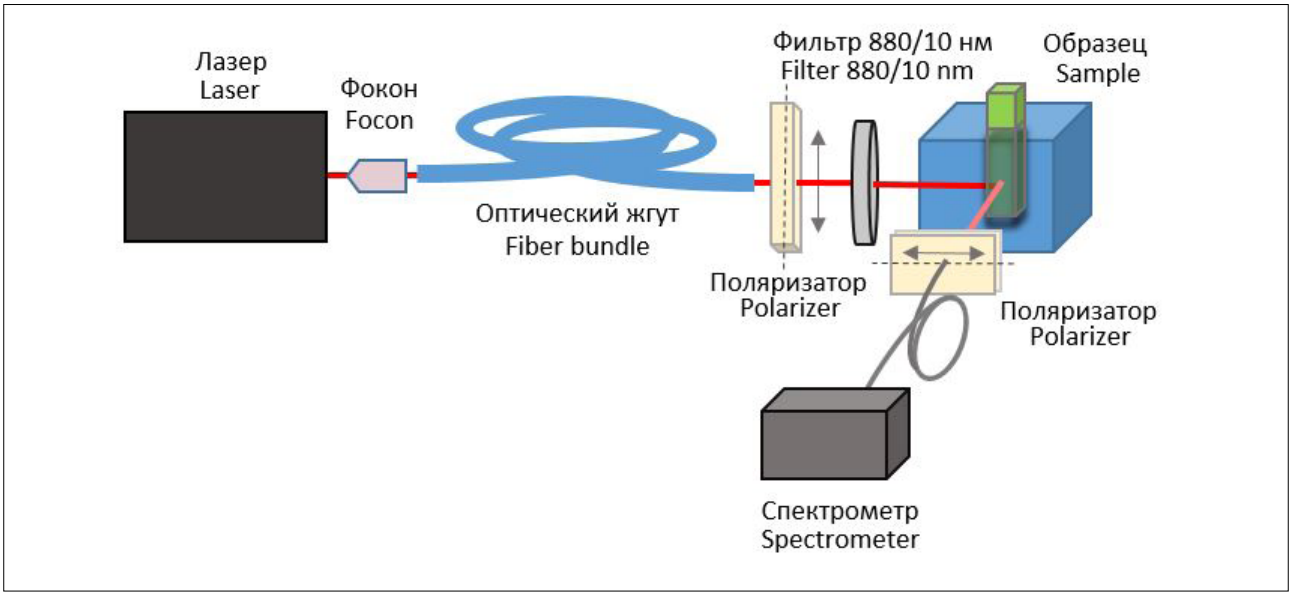


Рис. 2. Схема установки для определения спектроскопических свойств J-агрегатов ICG NPs и калибровочных сфер методом рассеяния света.
Fig. 2. Installation scheme for determination of spectroscopic properties of ICG NPs J-aggregates and calibration spheres by light scattering method.

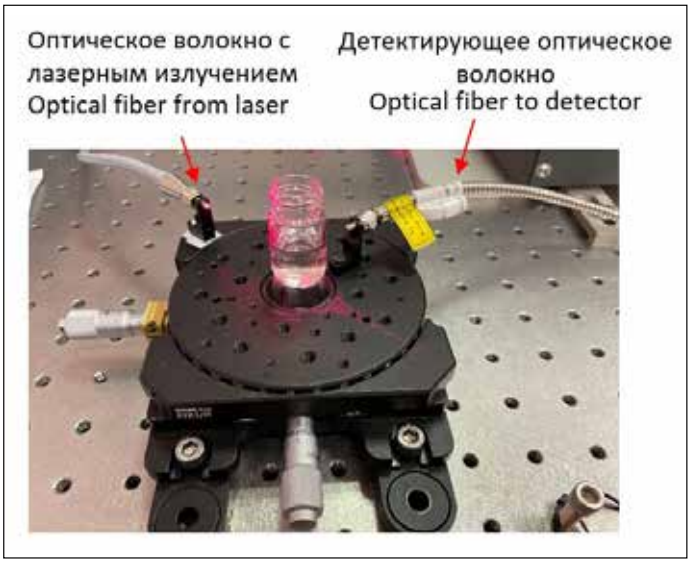


Рис. 3. Экспериментальная установка для определения индикатрисы рассеяния излучения, прошедшего через коллоидный раствор ICG NPs и образца, содержащего калибровочные сферы.
Fig. 3. Experimental installation for determining the scattering indicatrix of radiation passed through the colloidal solution of ICG NPs and the sample containing calibration spheres.

The experimental results were approximated by Henyey–Greenstein function to calculate the anisotropy factor:

$$p(cos\theta) = \frac{1-g^2}{2\left(1+g^2-2g*cos\theta\right)^{3/2}}, \quad (1)$$

where p – phase scattering function, θ – deflection angle of scattered light, g – anisotropy factor.

In the decomposition of the Henyey–Greenstein function by Legendre polynomials, the anisotropy factor is related to the expansion coefficients through the relation:

$$x_i = (2i+1)g^i, \quad (2)$$

The elongation of the scattering indicatrix is determined by this parameter.

To study the aggregate size and the aggregation degree of ICG molecules at different concentrations of ICG NPs colloidal solution, a multi-angle dynamic light scattering analyzer Photocor Complex (Photocor, Russia) was used. A He–Ne laser with 632.8 nm wavelength was used as an excitation source. 15° and 145° angles were selected to measure the power of the scattered signal on the samples of ICG NPs colloidal solution (Fig. 4).

The aggregation degree β_m demonstrates the ranking index of ICG molecules in a aggregate and is determined by comparing the scattering efficiency with the calculations of Mie scattering. The aggregation degree can be represented in the forms:

$$\beta_m = N_m / N_{ag}, \quad (3)$$

where N_m – numerical density of ICG molecules, N_{ag} – numerical density of J-aggregates, and

$$\beta_m = \frac{f_{ag} S_{ag} N_m}{a_{ag}}, \quad (4)$$

where f_{ag} – the proportionality factor**,** also called J-aggregate absorption strength, S_{ag} – absorption cross section of aggregate, a_{ag} – average size of aggregate.

Differential cross-sections of Mie scattering at different angles of light scattering on the sample were calculated to obtain the values of the aggregation degree of ICG molecules. These values were then used to calculate the average size of aggregates at different concentrations. A detailed description of the experiment

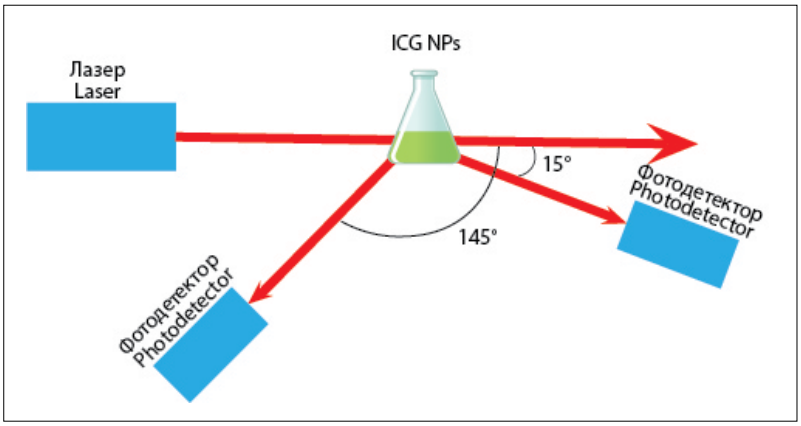


Рис. 4. Схема эксперимента измерения размера частиц методом рассеяния Ми.
Fig. 4. Experimental scheme of measuring the particle size by the Mie scattering method.

design and the theoretical derivation of the formulas are presented in work [11].

ICG NPs colloidal solution was examined by scanning electron microscopy (SEM) to study the morphological structure of J-aggregates. Before the study, samples of ICG NPs colloidal solution were frozen at 77 K (by immersion in liquid nitrogen). This method of samples temperature lowering was necessary to avoid aggregates sticking together and/or rearranging self-assembly. The pre-frozen sample was placed on the Peltier table (thermoelectric cooling) and placed in the chamber of the electron microscope EVO LS10 (Zeiss, Germany). The surface of the Peltier table was stabilized at 253 K temperature, the pressure in the chamber was 70 Pa. The Peltier table was heated from 77 K to 253 K in order to ensure the invariance of the component structure of ICG NPs samples. To avoid water screening, the surface of the ICG NPs sample droplets were subjected to preliminary electron beam effects until the structured image appeared. Observations were carried out in low vacuum mode at an 21 kV accelerating voltage and a current on the sample of 30 pA via a backscattered electron detector using a working segment of 13-13.5 mm. 1024×768 px images were recorded with 7.7 nm/px hardware resolution.

Results

The sample was irradiated with 650, 700, 750 and 800 nm laser radiation to obtain fluorescence spectra for study the spectroscopic properties of ICG NPs colloidal solution with 0.25·μM concentration (Fig. 5a).

During the exposion to a sample of ICG NPs colloidal solution by laser radiation with wavelengths of 750 and 800 nm, an intense fluorescence peak corresponding to ICG NPs J-aggregates was observed. When the sample is excited with 650 and 700 nm wavelengths, in addition to fluorescence of J-aggregates, a small fluorescence peak corresponding to ICG monomers is observed at 815 nm. It was noted that with an increase of laser radiation wavelength, a shift of the fluorescence peak to the short-wavelength region is observed, at the same time the dependence of the fluorescence peak and the wavelength is linear.

Fig. 5b shows the dependence of the fluorescent signal intensity (displayed in color) on the excitation wavelength for ICG NPs colloidal solution. The maximum fluorescence intensity of ICG NPs J-aggregates is observed with laser radiation with wavelengths in the range of 775-800 nm. There is also a fluorescence shift in of ICG NPs J-aggregates to the short-wave region with an increase in the wavelength of laser radiation (for clarification, a vertical dashed line at 900 nm wavelength is drawn).

In the course of the work, the J-aggregates fluorescence signal of ICG NPs colloidal solution under resonant excitation was also studied. A sample with a ICG NPs colloidal solution with a 0.25 μM concentration was irradiated with different wavelengths in 880-915 nm range. Polylatex calibration spheres with 110 nm diameter were used as a reference sample to obtain the instrument response function of the assembled system and assess polarizer system imperfections. The spectra of scattered laser radiation on samples of ICG NPs colloidal solution and calibration spheres in 880-915 nm wavelength range with 1 nm adjustment tuning step were obtained (Fig.6). The dependences of the intensity maxima of radiation

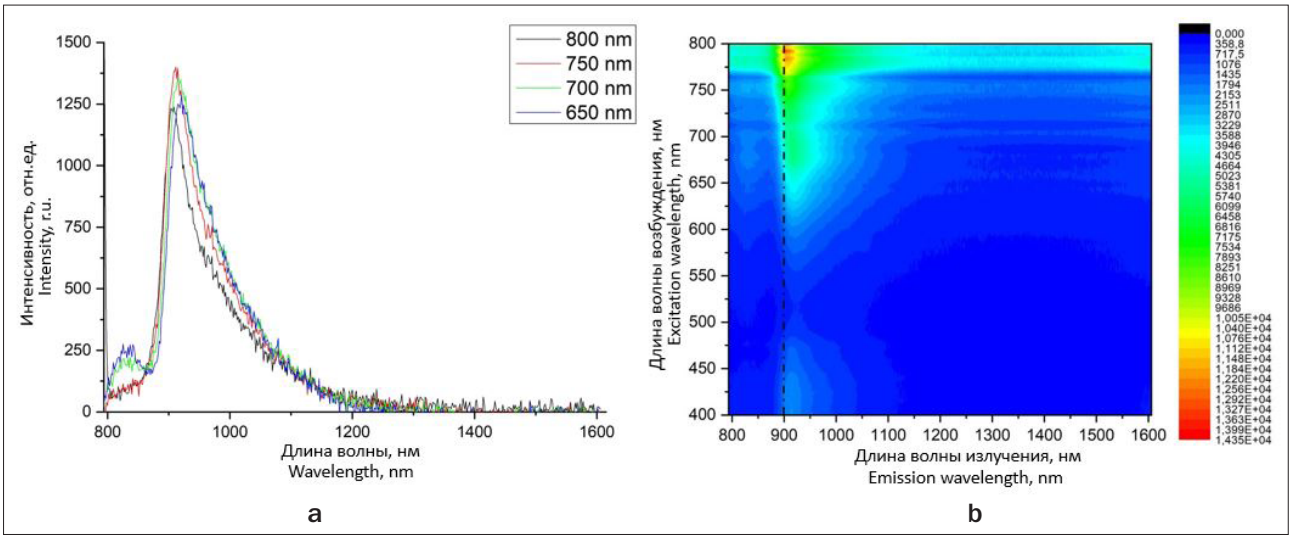


Рис. 5.
a – спектры флуоресценции J-агрегатов коллоидного раствора ICG NPs при возбуждении на 650, 700, 750 и 800 нм;
b – спектральная карта флуоресценции нормированных интенсивностей испускания флуоресценции при различных длинах волн.
Fig. 5.
a – fluorescence spectra of J-aggregates of ICG NPs colloidal solution at 650, 700, 750 and 800 nm excitation;
b – spectral map of normalized fluorescence emission intensities at different wavelengths.

scattered on samples of ICG NPs colloidal solution and calibration spheres were obtained for visual observation of changes in the intensity of scattered laser radiation (Fig.7a).

The decrease of the laser light scattered by the sample is observed with an increase of the laser radiation wavelength (Fig. 7a). The intensity maxima ratio of the scattered laser radiation from ICG NPs sample to a calibration sphere sample was obtained for evaluation of the spectroscopic properties of the ICG NPs colloidal solution (Fig. 7b). This dependence demonstrates the ability of ICG NPs colloidal solution to absorb in 893-896 nm range. Fig. 8 shows the ratio of the intensity maxima values of the scattered laser signal on ICG NPs colloidal solution to the calibration sphere sample, which shows the absorption caused by J-aggregates. To confirm this effect, absorption spectra were obtained via a two-

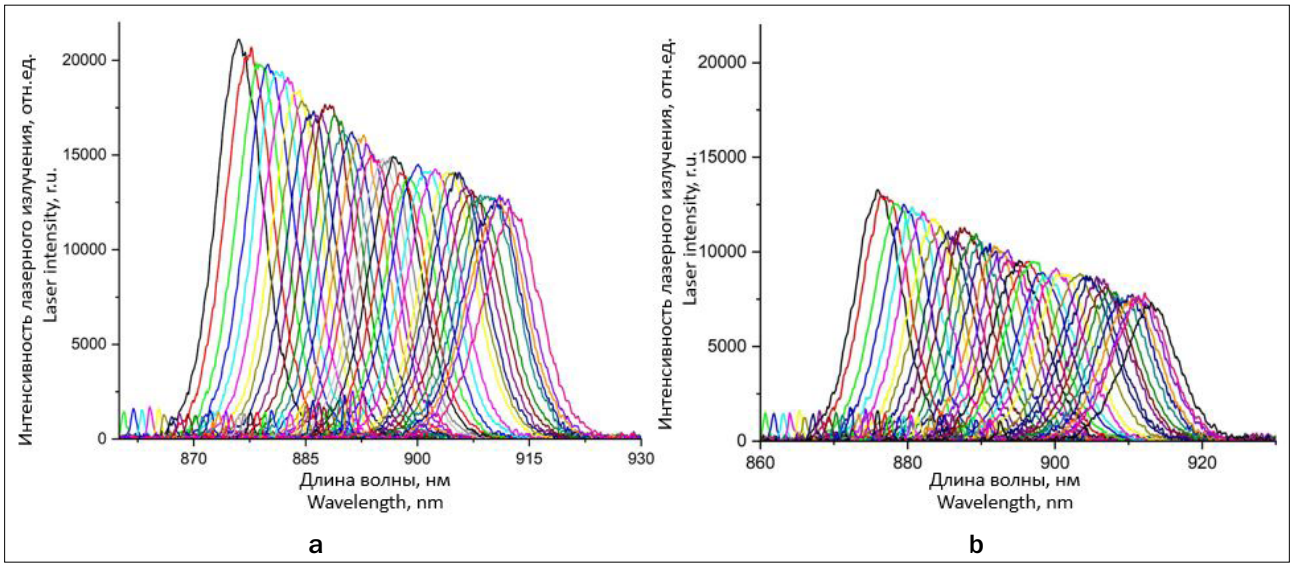


Рис. 6. Спектры рассеянного лазерного излучения, прошедшего через образец под 90°:
a – ICG NPs; b – калибровочные сферы
Fig.6. Spectra of scattered laser radiation transmitted through the sample at 90°:
a – ICG NPs; b – calibration spheres

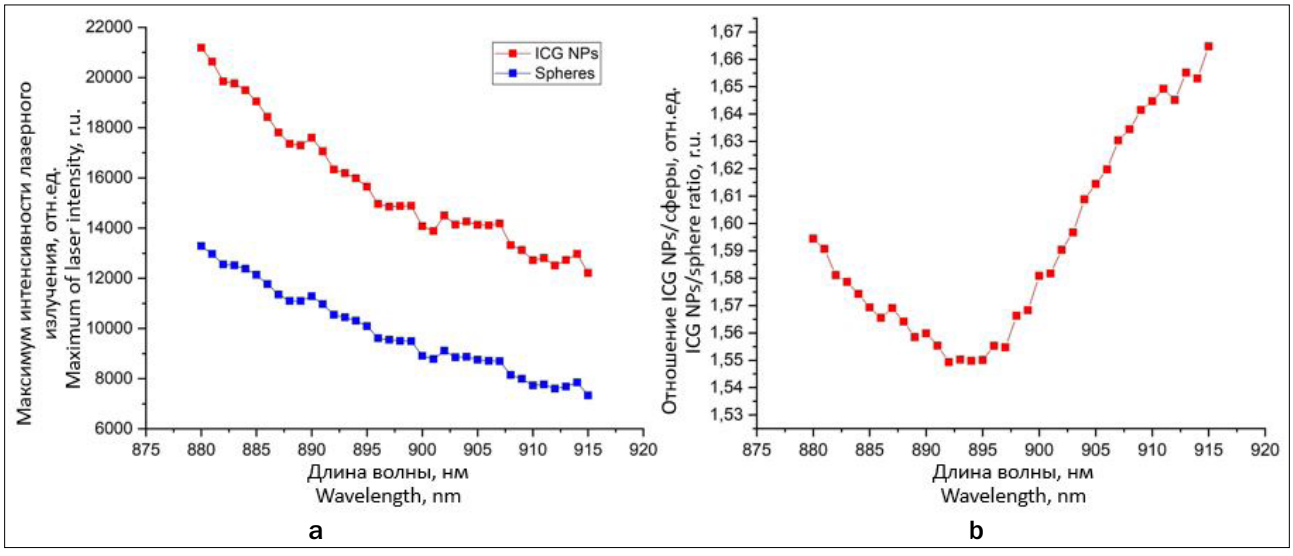


Рис. 7.
a – максимумы интенсивностей рассеянного излучения, прошедшего через образцы ICG NPs и калибровочные сферы в зависимости от длины волны лазерного излучения;
b – отношение максимумов интенсивностей рассеянного лазерного излучения, прошедшего через образец ICG NPs к калибровочным сферам.
Fig. 7.
a – intensity maxima of the radiation scattered by ICG NPs and calibration spheres depending on the laser radiation wavelength;
b – the ratio of the intensity maxima of the laser radiation scattered by ICG NPs sample to the calibration sphere sample.

beam spectrophotometer and a spectrometer when light passes through the sample to the lumen from a broadband source through ICG NPs colloidal solution and calibration spheres at 180°angle (Fig. 8).

According to the data obtained by two different methods, the J-aggregate maximum absorption of the ICG NPs colloidal solution is observed at 893 nm wavelength (Fig. 8).

To suppress the intensity of the laser radiation signal, a 880/10 nm cleaning narrowband filter was installed in front of the sample. Also, the spectra of the laser signal scattered on the samples were obtained with the configuration of the installation at 90° angle (Fig. 9).

Fig. 10a shows that a decrease in the maximum intensities of the scattered laser signal occurs with an increase in the wavelength of the laser radiation, while after 892 nm a low signal of scattered radiation is observed, which indicates the absorption of the laser signal by a narrow-band filter. The ratio of the intensity maxima of the scattered laser signal in Fig. 10b shows a decrease in the dependence values due to the absorption of J-aggregates of the ICG NPs colloidal solution and subsequent absorption by a narrowband filter of 880/10 nm.

The absorption spectra of J-aggregates when heating ICG NPs colloidal solution to a temperature of 65°C were used to plot a graph on a double logarithmic scale in order to estimate the aggregate number of nanoparticles [12]. From this dependence, based on the law of mass action:

$$K = \frac{C_{agg}}{C_m^n} \tag{5}$$

$$C_0 = C_m + nC_{agg} \tag{6}$$

$$C_0 - C_m = nC_{agg} = nKC_m^n \tag{7}$$

$$\lg nC_{agg} = \lg nK + n \lg C_m \tag{8}$$

where n – the monomer number forming the aggregate, K – proportionality coefficient, C_0 – total concentration of fluorescent dye, C_m – concentration of monomer ICG, C_{agg} – J-aggregate concentration. According to these equations, an aggregate number of ICG molecules was obtained equal to 4, which demonstrate spectroscopic properties corresponding to ICG NPs J-aggregates (Fig. 11). This result is confirmed by studies of the concentration dependences of the spectroscopic effect in work [13], assuming thermodynamic equilibrium between two ICG states: a monomer solution and J-aggregates. The aggregate number of molecules for H-aggregates of ICG is 2.

For obtaining a scattering indicatrix of light transmitted through samples of ICG NPs colloidal solution and calibration spheres, the installation shown in Fig. 3 was assembled with 920 nm wavelength. The data of the angular dependence of light scattering characterize the scattering properties of the object by their appearance, which allows comparing the shape and size of J-aggregates of ICG NPs colloidal solution with calibration spheres. Polar diagrams of the intensity dependence of scattered laser light on the rotation angle of the receiving fiber were obtained (Fig. 12).

The obtained data of polar diagrams of the light scattering indicatrix transmitted through a colloidal solution and calibration spheres are shown in Fig. 12, and demonstrate the change in the intensity of scattered light from the scattering angle. The scattering indicatrix of the light transmitted through the calibration spheres

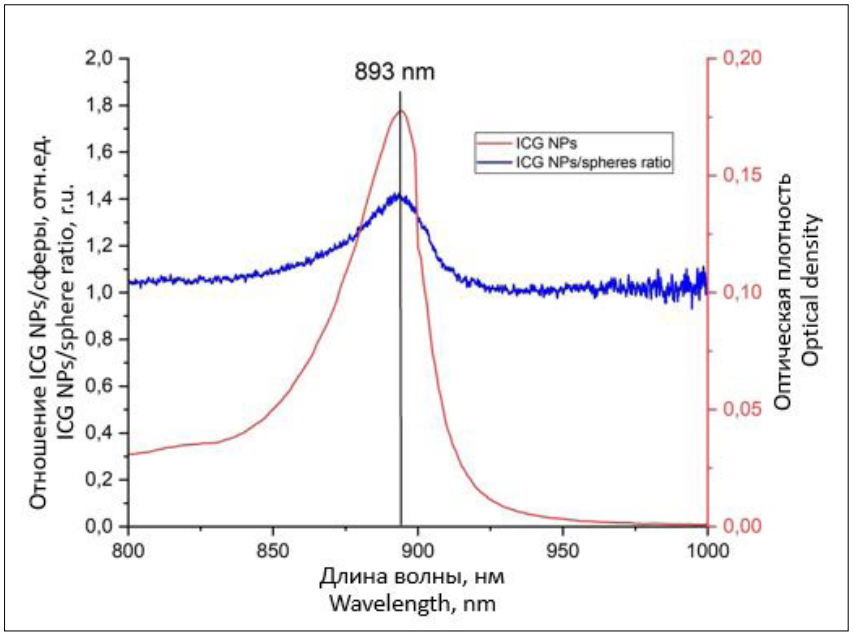


Рис. 8. Отношение спектров пропускания света через коллоидный раствор ICG NPs и через калибровочные сферы, полученных при помощи широкополосного источника света и волоконного спектрометра (синяя линия), спектр поглощения коллоидного раствора ICG NPs, полученный при помощи двухлучевого спектрофотометра (красная линия).
Fig. 8. The ratio of the light transmission spectra through the ICG NPs colloidal solution and the calibration spheres obtained via a broadband light source and a fiber spectrometer (blue line), the absorption spectrum of the ICG NPs colloidal solution obtained via a two-beam spectrophotometer (red line).

have a more elongated shape, which shows a cosine close to the one unit of the scattering angle compared to the ICG NPs colloidal solution. The calculated value of the g-factor according to the Henyey-Greenstein formula with 920 nm wavelength for the ICG NPs colloidal solution was 0.76, while for calibration spheres it was 0.86.

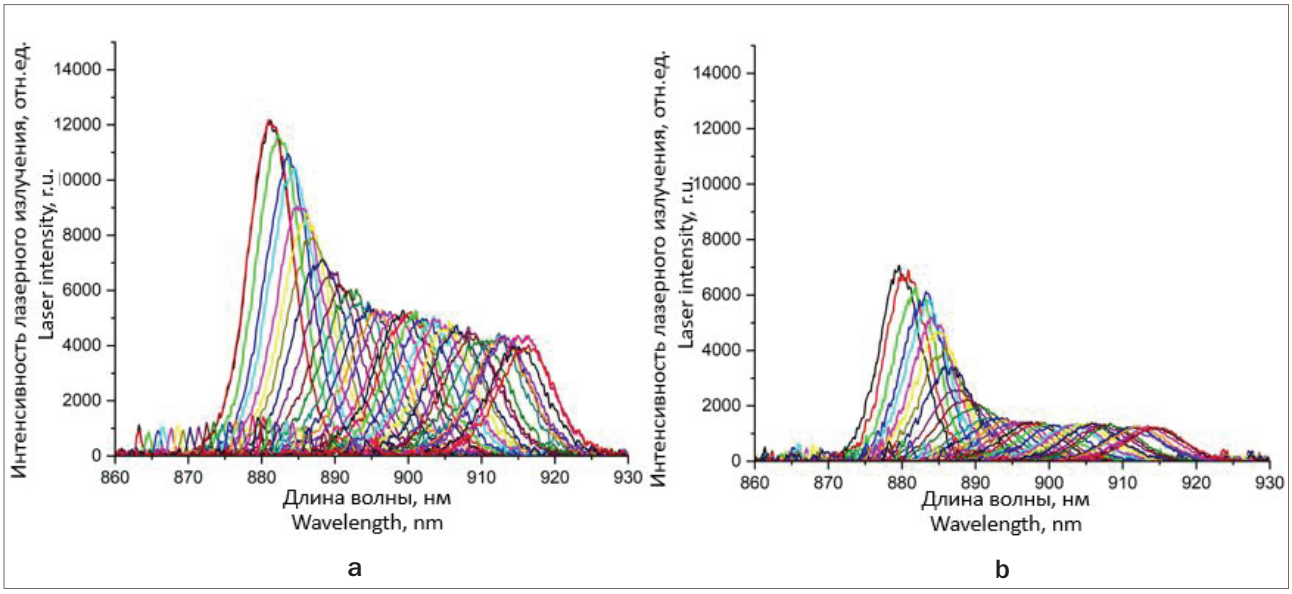


Рис. 9. Полученные спектры лазерного излучения, рассеянного на образцах под углом 90° с узкополосным фильтром 880/10 нм:
a – ICG NPs;
b – калибровочные сферы.
Fig. 9. The obtained spectra of laser radiation scattered on samples at 90° angle with 880/10 nm narrow-band filter:
a – ICG NPs;
b – calibration spheres.

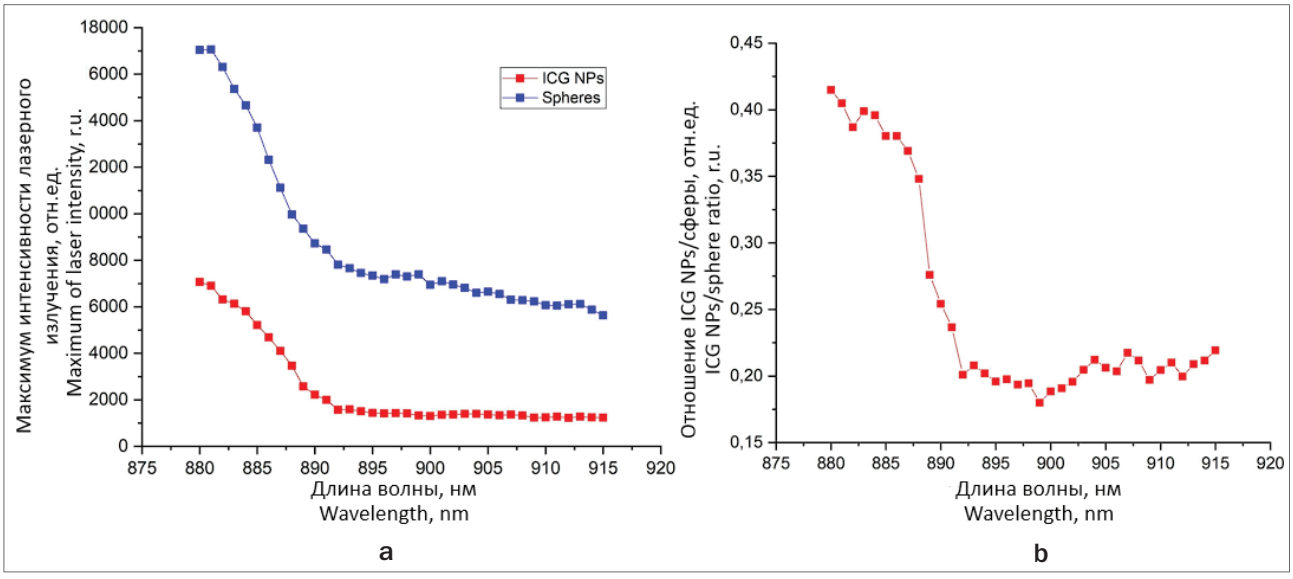


Рис. 10.
a – максимумы интенсивностей рассеянного лазерного излучения на образцах ICG NPs и на калибровочных сферах в зависимости от длины волны лазерного излучения;
b – отношение максимумов интенсивностей рассеянного лазерного излучения на образце ICG NPs и на калибровочных сферах.
Fig. 10.
a – intensity maxima of scattered laser radiation on samples of ICG NPs and the calibration spheres depending on the laser radiation wavelength;
b – the intensity maxim ratio of scattered laser radiation on the sample of ICG NPs and the calibration spheres.

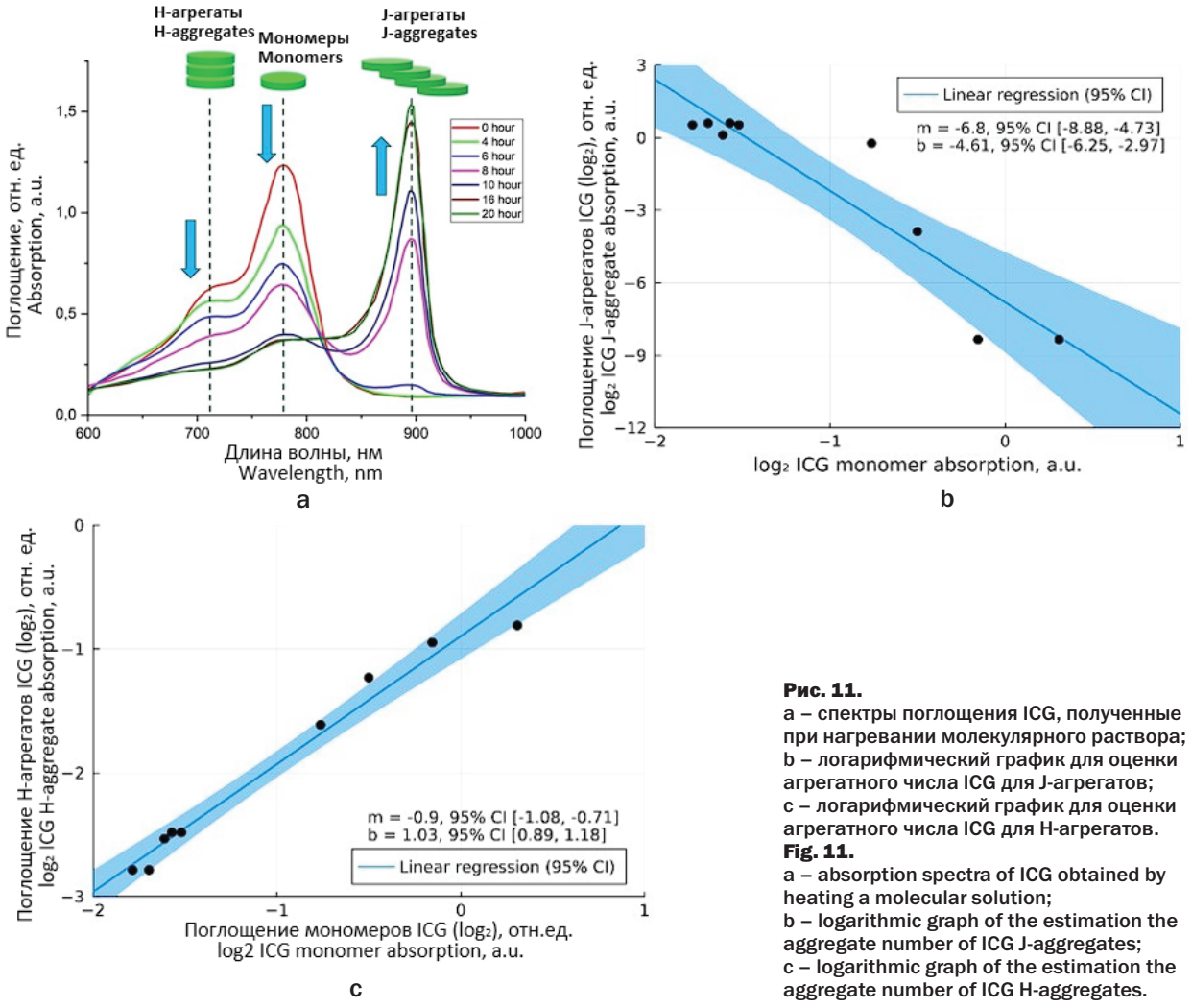


Рис. 11.
a – спектры поглощения ICG, полученные при нагревании молекулярного раствора;
b – логарифмический график для оценки агрегатного числа ICG для J-агрегатов;
c – логарифмический график для оценки агрегатного числа ICG для H-агрегатов.
Fig. 11.
a – absorption spectra of ICG obtained by heating a molecular solution;
b – logarithmic graph of the estimation the aggregate number of ICG J-aggregates;
c – logarithmic graph of the estimation the aggregate number of ICG H-aggregates.

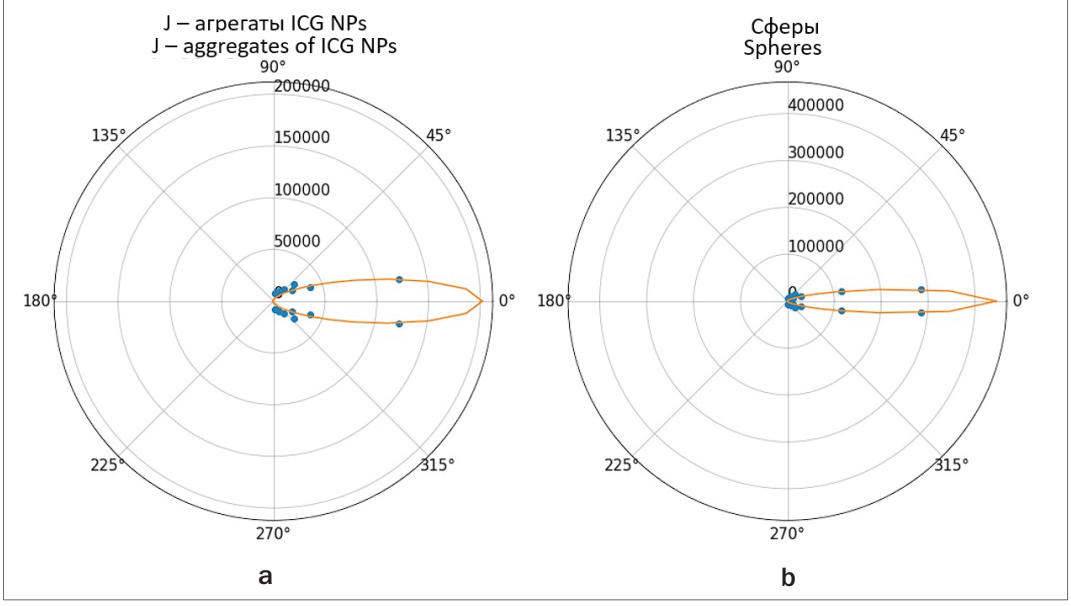


Рис. 12. Индикатрисы рассеяния света образцами:
a – коллоидный раствор ICG NPs;
b – калибровочные сферы.
Fig. 12. The scattering indicatrix of the by the samples:
a – ICG NPs colloidal solution;
b – calibration spheres.

For estimation the average size and aggregation degree of the J-aggregates of ICG NPs colloidal solution at different concentrations, the scattered light intensities on nanoparticles at 15° and 145° angles were measured by the Mie scattering method. On the basis of these data, the differential cross-sections of the Mie scattering are calculated as presented in study [11]. The experimentally obtained differential cross-sections of Mie scattering at θ = 15° and θ = 145° allowed us to determine the average aggregation degree and the average size of the J-aggregates of ICG NPs colloidal solution, respectively (Fig. 13a).

The aggregation degree of nanoparticles increases from 1.25×10^9 for 2·μM to 2.38×10^9 for 0.5 mM concentration of ICG NPs colloidal solution. Absorption spectra of ICG NPs colloidal solution were obtained from which the absorption coefficient of J-aggregates was calculated. Fig. 10b shows how the absorption coefficient of J-aggregates decreases with increasing concentrations, while the average size of J-aggregates increases, which varies from 144 nm for 2·μM to 175 nm for 0.5 mM concentration of ICG NPs colloidal solution. Presumably, until 75 μM, ICG NPs J-aggregates grow vertically by adding ICG molecules above and below by non–covalent π-π stacking, while after 75 μM, they increase in width by growing sideways and/or forming other ICG NPs J-aggregates.

Images of J-aggregates of ICG NPs colloidal solution were obtained by SEM method to study the morphological structure (Fig. 14).

Microscopic images show the position of ICG NPs J-aggregates in the form of large sheet-like conglomerates and in the form of flakes consisting of granular material, and with a wide size distribution. The J-aggregates of the ICG NPs colloidal solution are a tightly packed network of long rod-shaped conglomerates in the obtained images. Based on the obtained images, it is impossible to determine individual J-aggregates from beginning to end and the intersection points between individual aggregates, at the same time the J-aggregates are slightly curved. The formation of a network is the cause of the strong viscoelasticity observed in solutions. According to the obtained images, ICG NPs J-aggregates with 5·μM concentration are about 100-110 nm wide of the sheet-like aggregate and, presumably, more than 1 μm in length (Fig. 14a). With the concentration increase of the ICG

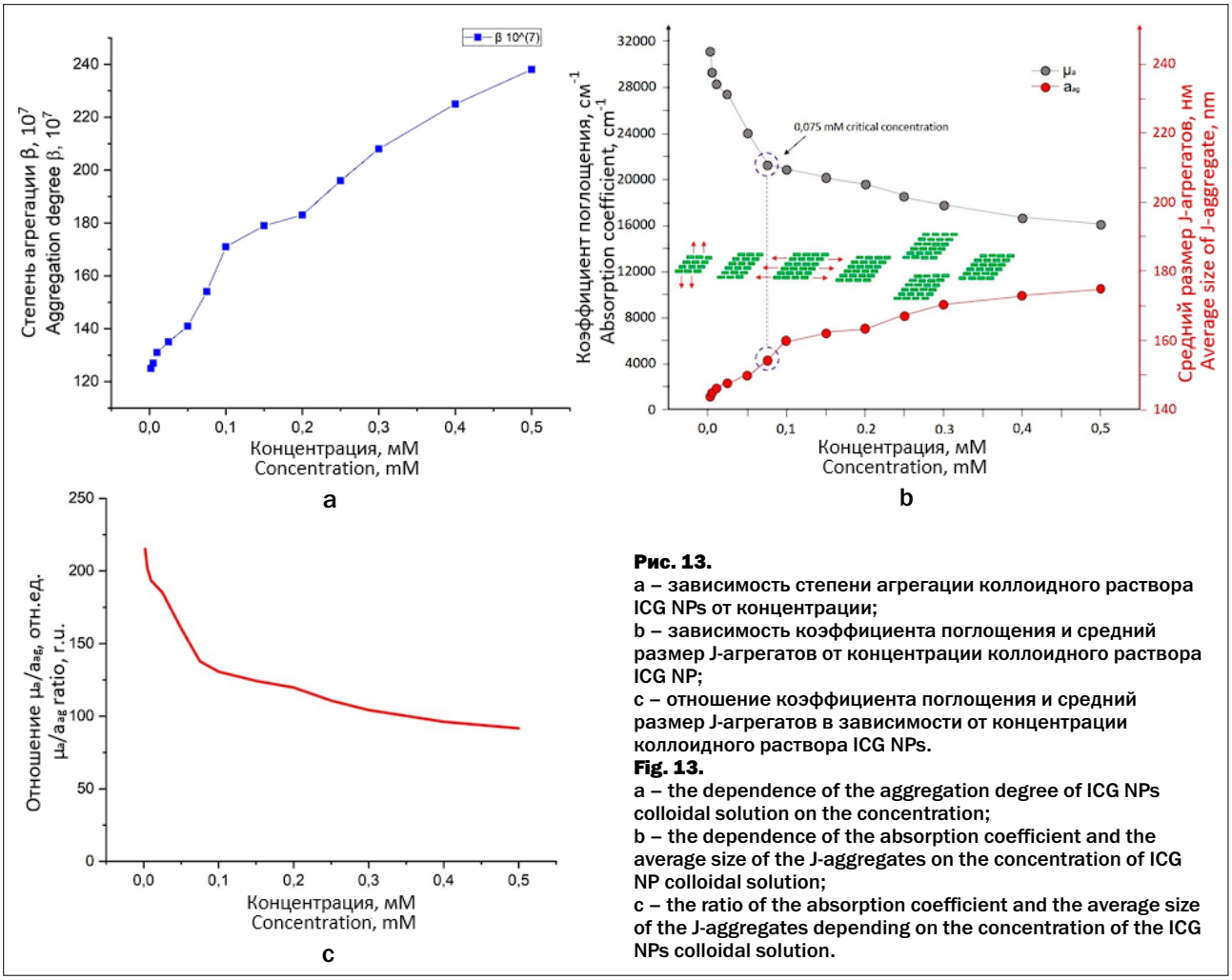


Рис. 13.
a – зависимость степени агрегации коллоидного раствора ICG NPs от концентрации;
b – зависимость коэффициента поглощения и средний размер J-агрегатов от концентрации коллоидного раствора ICG NP;
c – отношение коэффициента поглощения и средний размер J-агрегатов в зависимости от концентрации коллоидного раствора ICG NPs.

Fig. 13.
a – the dependence of the aggregation degree of ICG NPs colloidal solution on the concentration;
b – the dependence of the absorption coefficient and the average size of the J-aggregates on the concentration of ICG NP colloidal solution;
c – the ratio of the absorption coefficient and the average size of the J-aggregates depending on the concentration of the ICG NPs colloidal solution.

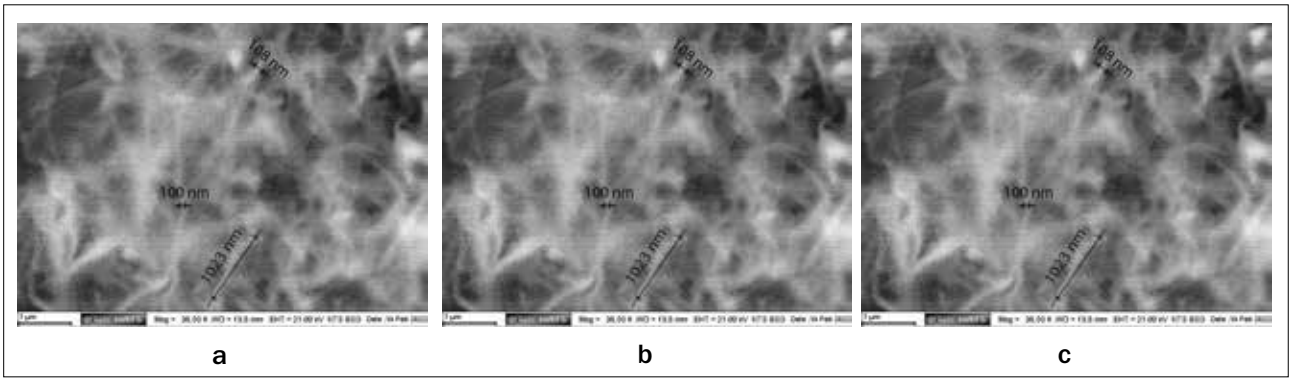


Рис. 14. SEM изображения J-агрегатов ICG NPs с концентрациями:
a – 5 μM; b – 0.1 мМ; c – 0.5 мМ.
Fig. 14. SEM images of ICG NPs J-aggregates with concentrations:
a – 5 μM; b – 0.1 mM; c – 0.5 mM.

NPs colloidal solution to 0.1 mM, the dimensions of the aggregates increase to 120 ± 5 nm in width and 1050 ± 15 nm in length (Fig. 14b). For higher concentrations than 0.5 mM, J-aggregates are large particles with a width up to 500 nm and a length up to 1 μm (Fig. 14c).

Discussion

In this work, the spectroscopic properties of ICG NPs colloidal solution, which mainly consists of J-aggregates were considered. A shift of the fluorescence maximum of ICG NPs J-aggregates to the short-wavelength range is noted with an increase in the wavelength of laser radiation. The spectral map of fluorescence upon excitation with different wavelengths of laser radiation demonstrates the broadening of the emission spectrum of ICG NPs J-aggregates with increasing fluorescence intensity.

During the study of scattered laser radiation on ICG NPs colloidal solution passing through crossed linear polarizers, a laser radiation signal was observed. The resonant fluorescence of ICG NPs colloidal solution turned out to be less intense compared to the scattered laser radiation, and the quality of the polarizer and the analyzer system did not allow to register a fluorescent signal.

Spectroscopic methods were used to obtain the minimum number of ICG molecules lined up in the brickwork of the ICG NPs J-aggregate, equal to 4, for the bathochromic shift of the optical properties of ICG NPs colloidal solution. When exposed to electromagnetic radiation, the upper and lower molecules rise on one side, breaking the bonds of interaction between the molecules (Fig. 15). The proposed configuration of molecules in the ICG NPs J-aggregate is potentially consistent with the data obtained from the law of mass action (Eq. 5-8).

Scattered laser radiation was recorded on a J-aggregates sample of ICG NPs colloidal solution by transverse scanning with a receiving fiber at different scattering angles to study the scattering indicatrix. It was noted that the efficiency of the scattered laser signal increases with decreasing scattering angle. The scattering indicatrix of light passing through the ICG NPs colloidal solution has an elongated forward shape, which is confirmed by the anisotropy g-factor calculated using the Henyey-Greenstein formula, equal to 0.76.

The aggregation degree and the average size of J-aggregates were investigated with increasing concentration of ICG NPs colloidal solution. The scattered signal at an 15° angle provided information on the average aggregation degree of the nanoparticles, while the scattered signal at an 145° angle provided information of the J-aggregates average size. A polydisperse size distribution of aggregates was noted, with increasing concentration increasing the J-aggregate size of ICG NPs and their aggregation degree, while the absorption coefficient decreases, which is consistent with theoretical studies [15]. The decrease in the absorption coefficient of J-aggregates is caused by a decrease in the total specific surface of molecules with an increase in their size [16, 17]. ICG monomers are added mainly from above and below the J-aggregate and slowly across up to a critical colloidal solution concentration of 75 μM. After that, J-aggregates begin to slowly build monomers in height and contribute to the addition of ICG molecules on the sides and/or form new J-aggregates.

In SEM images, ICG NPs J-aggregates are densely packed molecules with a sheet-like morphology, which is consistent with the brickwork packing model of ICG molecules proposed by Kuhn et al [18]. At the same time, with an increase in the concentration of the ICG NPs colloidal solution, a change in the structural morphology of the J-aggregate occurs, which is demonstrated by the addition of ICG monomers to the surface of the aggregates.

Based on the obtained data, a new model of the structural arrangement of molecules in the ICG J-aggregate under the action of laser radiation was proposed. The angle between the transition dipoles and the molecular axis of the aggregate determines whether the transition

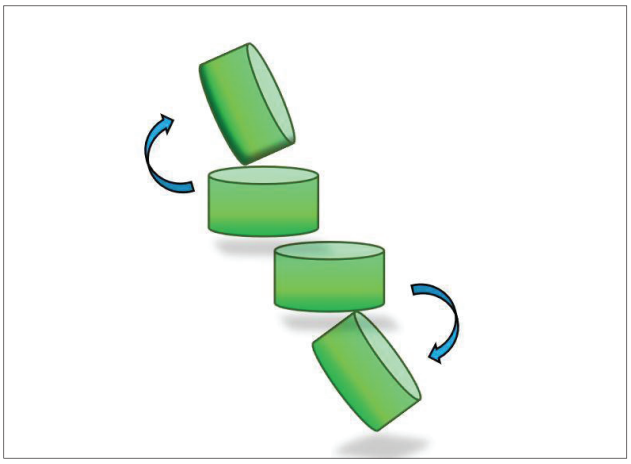


Рис. 15. Схематическое изображение активации молекул в J-агрегате ICG NPs при воздействии лазерного излучения.
Fig. 15. Schematic representation of the activation of molecules in the ICG NPs J-aggregate under the laser radiation.

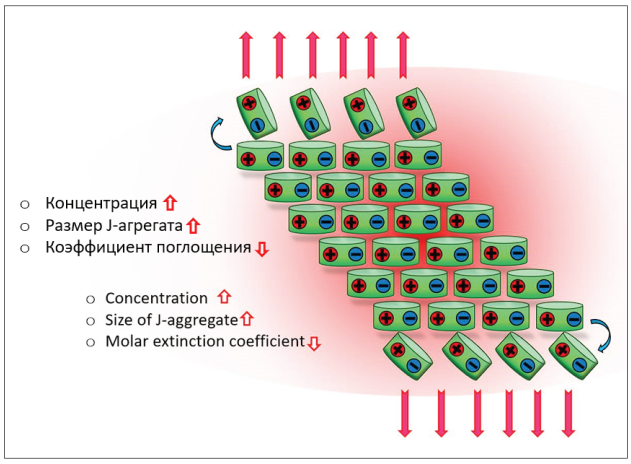


Рис. 16. Структурное расположение молекул в J-агрегате ICG NPs при воздействии лазерного излучения.
Fig. 16. Structural arrangement of molecules in the ICG NPs J-aggregate under the laser radiation.

to lower or higher levels of the excited state is allowed. In J- and H-type ICG aggregates, the excited state splits into two nondegenerate states. The low energy state is formed in the J-type ICG, which corresponds to codirectionally oriented transition dipole moments, while the high state contains transition dipoles with opposite orientations. In J-aggregates, only transitions to the lower level of the split excited state, which corresponds to the bathochromic spectral shift, are possible. J-aggregates of ICG NPs colloidal solution are arranged in monomolecular layers of molecules, the long axes of which lie parallel to the plane of the layer (Fig. 16). The shift of the absorption peak to the long wavelength region of the J-aggregate is due to the large lateral shift along the long molecular axis between adjacent ICG monomers. The dipole moments of the electronic transitions of individual molecules in the aggregate are aligned parallel to the line connecting their centers through the head-tail arrangement of the monomers. When exposed to electromagnetic radiation in this configuration, interactions occur with molecules located on the surface of the J-aggregate, which rise, breaking one interaction bond with the molecules of the aggregate with a greatly increased dipole moment (Fig. 16).

Resonant excitation occurs on the surface molecules of the J-aggregate. The narrow fluorescence spectrum is justified by the simultaneous uplift of molecules on the surface of the aggregate. As the concentration increases, a quasi-two-dimensional superstructure of ICG monomers occurs on the surface of the J-aggregate. The decrease in the absorption coefficient with increasing concentration is characterized by an increase in the probability of molecules transition to an excited state due to an increase in the number of molecules that make up ICG NPs J-aggregate. At the same time, J-aggregates are characterized by a wide size distribution, which varies from small oligomer particles to sheet-like aggregates hundreds of nanometers long.

Conclusion

This paper presents the optical characteristics of ICG NPs colloidal solution, mainly consisting of J-aggregates. The obtained scattering indicatrix and anisotropy factor give an idea of the size of the ICG aggregate shape. According to the obtained characteristics by Mie scattering methods, a change in spectral characteristics was noted with an increase in the concentration of ICG NPs colloidal solution. The aggregation degree and the average size of the J-aggregates of ICG NPs colloidal solution increases with increasing concentration, while the absorption coefficient decreases, which is associated with a decrease in the specific surface area with an increase in the size of the J-aggregates. The SEM study showed a sheet-like morphology of J-aggregates with subsequent attachment of ICG monomers to the surface of the aggregate with increasing concentration. Based on the obtained data, a behavior model of ICG monomers that are part of the J-aggregate was formulated. The upper and lower molecules located on the domain are able to move from para-position to ortho-position, demonstrating fluorescent properties with a greatly increased oscillator strength, which is associated with an increase in the radiation velocity. The use of ICG NPs colloidal solution in clinical practice will improve the efficiency of fluorescent diagnostics of tumor tissue, the boundaries of its growth and the pathways of metastasis.

Acknowledgment

The reported study was funded by RFBR according to the research project No. 21-52-15025 and partly by IEA (International Emerging Action) CNRS grant 00534.

REFERENCES

1. Shakiba M., Ng K.K., Huynh E., Chan H., Charron D.M., Chen J., Muhanna N., Foster F.S., Wilson B.C. and Zheng G. Stable J-aggregation enabled dual photoacoustic and fluorescence nanoparticles for intraoperative cancer imaging // Nanoscale. – 2016. – 8. – P.12618-12625. https://doi.org/10.1039/C5NR08165C
2. Zweck J. and Penzkofer A. Microstructure of indocyanine green J-aggregates in aqueous solution // Chemical Physics. – 20010. – 269. P.399-409. https://doi.org/10.1016/S0301-0104(01)00368-8
3. Bricks J.L., Slominskii Y.L., Panas I.D. and Demchenko A.P. Fluorescent J-aggregates of cyanine dyes: basic research and applications review // Methods and applications in fluorescence. – 2017. – 6, P.012001.
4. Obara Y., Saitoh K., Oda M. and Tani T. Room-temperature fluorescence lifetime of pseudoisocyanine (PIC) J excitons with various aggregate morphologies in relation to microcavity polariton formation // International Journal of Molecular Sciences. -2012. – 13. P.5851-5865. https://doi.org/10.3390/ijms13055851
5. Hill T.K., Abdulahad A., Kelkar S.S., Marini F.C., Long T.E., Provenzale J.M. and Mohs A.M. Indocyanine green-loaded nanoparticles for image-guided tumor surgery // Bioconjugate chemistry. – 2015. – 26. P.294-303. https://doi.org/10.1021/bc5005679
6. Wittmann M., Rotermund F., Weigand R. and Penzkofer A. Saturable absorption and absorption recovery of indocyanine green J-aggregates in water // Applied Physics B: Lasers &Optics. – 1998. – 66.
7. Würthner F., Kaiser T.E. and Saha-Möller C.R. J-aggregates: from serendipitous discovery to supramolecular engineering of functional dye materials // Angew. Chem., Int. Ed. – 2011. -50. P.3376–410. https://doi.org/10.1002/anie.201002307
8. Farrakhova D., Maklygina Y., Romanishkin I., Yakovlev D., Plyutinskaya A., Bezdetnaya L. and Loschenov V. Fluorescence imaging analysis of distribution of indocyanine green in molecular and nanoform in tumor model // Photodiagnosis and Photodynamic Therapy. – 2022. – 37. P.102636. https://doi.org/10.1016/j.pdpdt.2021.102636
9. Farrakhova D., Romanishkin I., Maklygina Y., Bezdetnaya L. and Loschenov V. Analysis of Fluorescence Decay Kinetics of Indocyanine Green Monomers and Aggregates in Brain Tumor Model In Vivo // Nanomaterials. – 2021. – 11, P.3185. https://doi.org/10.3390/nano11123185
10. Farrakhova D.S., Romanishkin I.D., Yakovlev D.V., Maklygina Yu.S., Savelieva T.A., Bezdetnaya L., Loschenov V.B. The spectroscopic study of indocyanine green J-aggregate stability in human blood and plasma // Physics of Wave Phenomena. – 2022. – 30. P.86-90. https://doi.org/10.3103/S1541308X22020029
11. Weigand R., Rotermund F. and Penzkofer A. Degree of aggregation of indocyanine green in aqueous solutions determined by Mie scattering // Chemical physics. – 1997. – 220. 373-P.384. https://doi.org/10.1016/S0301-0104(97)00150-X
12. Liu R., Tang J., Xu Y., Zhou Y., Dai Z. Nano-sized indocyanine green J-aggregate as a one-component theranostic agent // Nanotheranostics. – 2017. – 1. P.430. https://doi.org/10.7150/ntno.19935
13. Wang J., Pang X., Tan X., Song Y., Liu L., You Q., Sun Q., Tan F., Li N. A triple-synergistic strategy for combinational photo/radiotherapy and multi-modality imaging based on hyaluronic acid-hybridized polyaniline-coated WS2 nanodots // Nanoscale. – 2017. – 9. P.5551-5564. https://doi.org/10.1039/C6NR09219E
14. Berlepsch H.V. and Böttcher C. Cryo-transmission electron microscopy reveals mesoscopic H-and J-aggregates of near infrared cyanine dyes // Journal of Photochemistry and Photobiology A: Chemistry. – 2010. -214. P.16-21. https://doi.org/10.1016/j.jphotochem.2010.05.025
15. Weigand R., Rotermund F. and Penzkofer A. Aggregation dependent absorption reduction of indocyanine green // The Journal of Physical Chemistry A. – 1997. – 101. P.7729-7734. https://doi.org/10.1021/jp9700894
16. Gregg S.D. and Sing K.S.W. Adsorption, Surface Area and Porosity // Journal of The electrochemical society. – 1967. – 114. P.279Ca.
17. Lowell S. and Shields J. E. Powder surface area and porosity // Springer Science & Business Media. – 1991. – 2.
18. Czikkely V., Försterling H.D. and Kuhn H. Light absorption and structure of aggregates of dye molecules // Chem. Phys. Lett. – 1970. – 6. P.11–14. https://doi.org/10.1016/0009-2614(70)80062-8

ЛИТЕРАТУРА

1. Shakiba M., Ng K.K., Huynh E., Chan H., Charron D.M., Chen J., Muhanna N., Foster F.S., Wilson B.C. and Zheng G. Stable J-aggregation enabled dual photoacoustic and fluorescence nanoparticles for intraoperative cancer imaging // Nanoscale. – 2016. – 8. – P.12618-12625. https://doi.org/10.1039/C5NR08165C
2. Zweck J. and Penzkofer A. Microstructure of indocyanine green J-aggregates in aqueous solution // Chemical Physics. – 2001. – 269. P.399-409. https://doi.org/10.1016/S0301-0104(01)00368-8
3. Bricks J.L., Slominskii Y.L., Panas I.D. and Demchenko A.P. Fluorescent J-aggregates of cyanine dyes: basic research and applications review // Methods and applications in fluorescence. – 2017. – 6, P.012001.
4. Obara Y., Saitoh K., Oda M. and Tani T. Room-temperature fluorescence lifetime of pseudoisocyanine (PIC) J excitons with various aggregate morphologies in relation to microcavity polariton formation // International Journal of Molecular Sciences. -2012. – 13. P.5851-5865. https://doi.org/10.3390/ijms13055851
5. Hill T.K., Abdulahad A., Kelkar S.S., Marini F.C., Long T.E., Provenzale J.M. and Mohs A.M. Indocyanine green-loaded nanoparticles for image-guided tumor surgery // Bioconjugate chemistry. – 2015. – 26. P.294-303. https://doi.org/10.1021/bc5005679
6. Wittmann M., Rotermund F., Weigand R. and Penzkofer A. Saturable absorption and absorption recovery of indocyanine green J-aggregates in water // Applied Physics B: Lasers &Optics. – 1998. – 66.
7. Würthner F., Kaiser T.E. and Saha-Möller C.R. J-aggregates: from serendipitous discovery to supramolecular engineering of functional dye materials // Angew. Chem., Int. Ed. – 2011. -50. P.3376–410. https://doi.org/10.1002/anie.201002307
8. Farrakhova D., Maklygina Y., Romanishkin I., Yakovlev D., Plyutinskaya A., Bezdetnaya L. and Loschenov V. Fluorescence imaging analysis of distribution of indocyanine green in molecular and nanoform in tumor model // Photodiagnosis and Photodynamic Therapy. – 2022. – 37. P.102636. https://doi.org/10.1016/j.pdpdt.2021.102636
9. Farrakhova D., Romanishkin I., Maklygina Y., Bezdetnaya L. and Loschenov V. Analysis of Fluorescence Decay Kinetics of Indocyanine Green Monomers and Aggregates in Brain Tumor Model In Vivo // Nanomaterials. – 2021. – 11, P.3185. https://doi.org/10.3390/nano11123185
10. Farrakhova D.S., Romanishkin I.D., Yakovlev D.V., Maklygina Yu.S., Savelieva T.A., Bezdetnaya L., Loschenov V.B. The spectroscopic study of indocyanine green J-aggregate stability in human blood and plasma // Physics of Wave Phenomena. – 2022. – 30. P.86-90. https://doi.org/10.3103/S1541308X22020029
11. Weigand R., Rotermund F. and Penzkofer A. Degree of aggregation of indocyanine green in aqueous solutions determined by Mie scattering // Chemical physics. – 1997. – 220. 373-P.384. https://doi.org/10.1016/S0301-0104(97)00150-X
12. Liu R., Tang J., Xu Y., Zhou Y., Dai Z. Nano-sized indocyanine green J-aggregate as a one-component theranostic agent // Nanotheranostics. – 2017. – 1. P.430. https://doi.org/10.7150/ntno.19935
13. Wang J., Pang X., Tan X., Song Y., Liu L., You Q., Sun Q., Tan F., Li N. A triple-synergistic strategy for combinational photo/radiotherapy and multi-modality imaging based on hyaluronic acid-hybridized polyaniline-coated WS2 nanodots // Nanoscale. – 2017. – 9. P.5551-5564. https://doi.org/10.1039/C6NR09219E
14. Berlepsch H.V. and Böttcher C. Cryo-transmission electron microscopy reveals mesoscopic H-and J-aggregates of near infrared cyanine dyes // Journal of Photochemistry and Photobiology A: Chemistry. – 2010. -214. P.16-21. https://doi.org/10.1016/j.jphotochem.2010.05.025
15. Weigand R., Rotermund F. and Penzkofer A. Aggregation dependent absorption reduction of indocyanine green // The Journal of Physical Chemistry A. – 1997. – 101. P.7729-7734. https://doi.org/10.1021/jp9700894
16. Gregg S.D. and Sing K.S.W. Adsorption, Surface Area and Porosity // Journal of The electrochemical society. – 1967. – 114. P.279Ca.
17. Lowell S. and Shields J. E. Powder surface area and porosity // Springer Science & Business Media. – 1991. – 2.
18. Czikkely V., Försterling H.D. and Kuhn H. Light absorption and structure of aggregates of dye molecules // Chem. Phys. Lett. – 1970. – 6. P.11–14. https://doi.org/10.1016/0009-2614(70)80062-8

PHOTODYNAMIC THERAPY OF PRIMARY AND RECURRENT FORMS OF WEAKLY PIGMENT CHOROIDAL MELANOMA

Zhyliayeva K.P., Demeshko P.D., Navumenka L.V., Krasny S.A., Tzerkovsky D.A., Zherko I.Yu.
N.N. Alexandrov National Cancer Center of Belarus, Lesnoy, Republic of Belarus

Abstract

Treatment of poorly-pigmented tumors of small sizes can be carried out using photodynamic therapy (PDT). The material for the analysis was data on 112 patients. We used data from the Belarusian Cancer Registry, medical records of patients with clinically diagnosed choroid melanoma (C69.3 according to ICD-10) for the period 2013–2021. The size and level of blood flow in the tumors were assessed using an ultrasound machine with a doppler attachment. PDT was carried out using a «UPL PDT» semiconductor laser (Lemt, Republic of Belarus, λ=661 nm) with a light spot diameter of 1 to 3 mm for 60 s per field with a light dose of 50 J/cm^2. The entire surface of the tumor was exposed to the action, with the fields "tiled", from the periphery to the top of the tumor, with overlapping fields. Tumor pigmentation was assessed visually. To evaluate the treatment outcome, the general group of patients was divided into three subgroups according to thickness and basal diameter. Group I – 40 (35.7%) patients, with an average tumor thickness of 1.4±0.2 mm, basal diameter – 5.8±1.5 mm. II – 51 (45.5%) patients, with an average tumor thickness of 2.3 ± 0.3 mm, basal diameter – 7.9 ± 1.5 mm. III – 21 (18.8%) patients. The mean value of the tumor thickness was 3.8±0.4 mm, the basal diameter was 9.8±1.4 mm. After PDT in the general group (n=112), 29 (25.9%) patients had complete tumor resorption, and 83 (74.1%) patients had stabilization. The eyeball was saved in 107 (95.5%) patients. Continued growth and relapse were recorded in 34 patients: 25 (22.3%) and 9 (8.0%), respectively. In 29 (85.3%) patients, the eyeball was preserved after treatment of relapse and continued growth. 5 (4.5%) enucleations were performed. Adjusted one-year cumulative survival was 100%, 3-year and 5-year 95.8±2.4%, 93.7±3.1%, respectively.

Key words: choroid melanoma, poorly pigmented tumor, photodynamic therapy, laser transpupillar thermotherapy, brachytherapy, recurrence.

For citations: Zhyliayeva K.P., Demeshko P.D., Navumenka L.V., Krasny S.A., Tzerkovsky D.A., Zherko I.Yu. Photodynamic therapy of primary and recurrent forms of weakly pigment choroidal melanoma, *Biomedical Photonics*, 2022, vol. 11, no. 3, pp. 17–23. doi: 10.24931/2413–9432–2022–11-3-17–23.

Contacts: Tzerkovsky D.A., e-mail: tzerkovsky@mail.ru

ФОТОДИНАМИЧЕСКАЯ ТЕРАПИЯ ПЕРВИЧНЫХ И РЕЦИДИВНЫХ СЛАБОПИГМЕНТНЫХ ФОРМ МЕЛАНОМЫ СОСУДИСТОЙ ОБОЛОЧКИ ГЛАЗА

Е.П. Жиляева, П.Д. Демешко, Л.В. Науменко, С.А. Красный, Д.А. Церковский, И.Ю. Жерко
Республиканский научно-практический центр онкологии и медицинской радиологии им. Н.Н. Александрова, п. Лесной, Республика Беларусь

Резюме

Лечение слабопигментных опухолей малых размеров может проводиться с применением фотодинамической терапии (ФДТ). Материалом для анализа послужили полученные из Белорусского канцер-регистра данные медицинской документации 112 пациентов с клинически установленным диагнозом меланомы сосудистой оболочки глаза (С69.3 по МКБ-10) за период 2013–2021 гг. Оценку размеров и уровня кровотока в опухолях осуществляли с использованием УЗИ аппарата с приставкой допплер. ФДТ проводили с использованием полупроводникового лазера «УПЛ ФДТ» (Lemt, Республика Беларусь, λ=661 нм) с диаметром светового пятна от 1 до 3 мм в течение 60 с на одно поле со световой дозой 50 Дж/см^2. Воздействию подвергали всю поверхность опухоли, располагая поля «черепицеобразно», от периферии к вершине опухоли, с перекрытием полей. Пигментацию опухоли оценивали визуально. Для оценки результата лечения общая группа пациентов была разделена на три подгруппы по толщине и базальному диаметру опухоли. I группа – 40 (35,7%) пациентов со средним значением толщины опухоли 1,4±0,2 мм и базальным диаметром 5,8±1,5 мм. II – 51 (45,5%) пациент со средним значением толщины опухоли 2,3±0,3 мм и базальным диаметром 7,9±1,5 мм. III группа – 21 (18,8%) пациент со средним значением толщины опухоли 3,8±0,4 мм и базальным диаметром 9,8±1,4 мм. После ФДТ в общей группе (n=112) у 29 (25,9%) зарегистрирована полная резорбция опухоли, у 83 (74,1%) стабилизация. Сохранить глазное яблоко удалось у 107 (95,5%) пациентов. Продолженный рост и рецидив регистрировался у 34 пациентов: 25 (22,3%) и 9 (8,0%), соответственно. У 29 (85,3%) пациентов сохранено глазное яблоко после лечения рецидива и продолженного роста. Произведено 5 (4,5%) энуклеаций Скорректированная одногодичная кумулятивная (СКВ) выживаемость составила 100%, 3-летняя и 5-летняя – 95,8±2,4%, 93,7±3,1%, соответственно.

Ключевые слова: меланома хориоидеи, слабопигментная опухоль, фотодинамическая терапия, лазерная транспупиллярная термотерапия, брахитерапия, рецидив, продолженный рост.

Для цитирования: Жиляева Е.П., Науменко Л.В., Красный С.А., Демешко П.Д. Церковский Д.А. Жерко И.Ю. Фотодинамическая терапия первичных и рецидивных слабопигментных форм меланомы сосудистой оболочки глаза // Biomedical Photonics. – 2022. – Т. 11, № 3. – С. 17–23. doi: 10.24931/2413–9432–2022–11-3-17–23.

Контакты: Церковский Д.А., e-mail: tzerkovsky@mail.ru

Introduction

Melanoma of the vascular membrane of the eye (choroidal melanoma, CM) is a tumor that develops from a clone of cells of the second pigment system (neural crest) which has a malignant potential. The average age of the patients is 64.0±10.0 years [1]. In the Republic of Belarus, the peak of morbidity has shifted to older age groups recently [2]. Almost half of the patients who seek help have large tumors and cannot be cured by organ-preserving treatments. Minimally invasive methods with preservation of the functions of the eyeball are preferable for the treatment of small tumors. Patients are cured with good results using laser techniques such as laser transpupillary thermotherapy (TTT), photodynamic therapy (PDT). In recent years, PDT has been actively developed [3] and has proven itself well in the treatment of CM and other malignant lesions of the membranes of the eyes, including osteomas and metastatic lesions [4]. According to some authors, PDT can be used before a biopsy to reduce the risk of bleeding from a tumor during an invasive method [5]. PDT gives encouraging results related to visual acuity compared with radiation therapy in the treatment of severe oncopathology of the eye membranes [4, 6, 7, 8]. CM develops from melanocytes and belongs to one of the most resistant tumors to all known methods of treatment. Studies conducted all over the world show encouraging results of the use of PDT in CM treatment [9].

Materials and methods

Database from the Belarusian Cancer Registry and case histories of patients with a clinically established diagnosis of CM (C 69.3 according to ICD-10) for the period of 2013-2021 are used in the study.

The research includes the study of 112 patients with weakly pigmented CM. 37 (33.0%) of the patients are men and the remaining are women75 (67.0%). Tumor pigmentation was assessed visually. All patients received PDT. The minimum age was 22 years, the maximum was 85 years, the median was 64.5±9.0 years. The average values of tumor thickness were 2.3±0.7 mm, basal diameter – 7.4±1.9 mm. 84 (75.0%) patients were diagnosed with a tumor with a prevalence of cT1N0M0, 28 (25.0%) – cT2N0M0. One course of PDT was used to treat 80 (71.4%) patients, 2 courses for 21 (18.8%) patients, 3 courses for 10 (8.9%) patients, and 1 (0.9%) patient underwent 4 courses of PDT. There were 93 (83.0%) patients with a single diagnosed CM. Synchronous and metachronous cancer was registered in the case of 19 (17.0%) patients.

For a detailed assessment of the results of the conducted treatment, the patients were divided into three groups depending on the thickness and basal diameter of the tumor.

Group I included 40 (35.7%) patients, with the number of 12 (30.0%) men and 28 (70.0%) women. The minimum age was 22 years, the maximum was 81 years, the median was 65.5±8.1 years. In the case of 38 (95.0%) patients, the prevalence of shingles was cT1N0M0, in 2 (5.0%) cases – cT2N0M0. The minimum thickness of pubescence is 0.8 mm, the maximum is 1.7 mm, the average value is 1.4 ± 0.2 mm. The minimum basal pubescence diameter is 1.2 mm, the maximum is 10.3 mm, the average value is 5.8 ± 1.5 mm. One course of PDT was applied to 34 (85.0%) patients, 2 courses – 5 (12.5%), 3 courses – 1 (2.5%). Synchronous and metachronous cancer was registered in the case of 5 (12.5%) patients.

Group II included 51 (45.5%) patients with the number of men – 16 (31.4%), women-35 (68.6%). The minimum age was 30 years, the maximum was 85 years, the median was 62.5±9.0 years. 42 patients (82.4%) were diagnosed with cT1N0M0 tumor, 9 patients (17.6%) – cT2N0M0. The minimum thickness of the tumor was 1.8 mm, the maximum was 3.0 mm, the average values were 2.3± 0.3 mm, the minimum size of the basal diameter of the tumor was 3.0 mm, the maximum was 11.9 mm, the average value was 7.9±1.5 mm. Synchronous and metachronous cancer was registered in the case of 7 (13.7%) patients. One course of PDT was applied to 31 (60.8%) patients, 2 courses – 13 (25.5%), 3 courses – 6 (11.8%), 4 courses – 1 (1.9%).

Group III included 21 (18.8%) patients with the number of men – 9 (42.9%), women – 12 (57.1%). The minimum age was 31 years, the maximum was 83 years, the median was 63.0±9.1 years. 4 (19.0%) patients were diagnosed with cT1N0M0 tumor, 17 (81.0%) – cT2N0M0. The minimum thickness of the tumor is 3.1 mm, the maximum is 5.2 mm, the average values were 3.8± 0.4 mm, the minimum size of the basal diameter of the tumor was 7.0 mm, the maximum was 13.5 mm, the average

value was 9.8±1.4 mm. One course of PDT was applied to 15 (71.4%) patients, 2 courses – 3 (14.3%), 3 courses – 3 (14.3%). Synchronous and metachronous cancer was registered in 7 (33.3%).

The size and level of blood flow in the tumors were evaluated using ultrasound with the Doppler prefix. Photolon (RUE "Belmedpreparaty", the Republic of Belarus) was used as a photosensitizer, which was administered intravenously for 30 minutes at a dose of 2.0–2.5 mg/kg of the patient's body weight in a darkened room 3 hours before PDT. A semiconductor laser "UPL PDT" (Lemt, Republic of Belarus, λ=661 nm) with a light spot diameter from 1 to 3 mm was used for 60 seconds per field with a light dose of 50 J/cm2. The entire surface of the tumor was exposed, placing the fields "tile-like", from the periphery to the top of the tumor, with overlapping fields.

Table 1 shows the distribution of tumor in groups depending on the localization on the fundus.

The immediate result of treatment was evaluated according to the WHO recommendation for solid tumors. Complete resorption of the tumor was characterized by the formation of a full-fledged focus of atrophy in the area of the former occurrence of the tumor, however, possible dispersion or a slight accumulation of pigment was allowed. The decrease in the size of the tumor, the absence of size changes in the case of the pronounced pigmentation and the absence of blood flow were considered to be the main criteria for stabilizing the tumor process. The ineffectiveness of the treatment consisted in the absence of tumor changes or the increase in its size with the preservation or enhancement of blood flow in it. The positive result of treatment was considered complete resorption or stabilization of the tumor process.

During dynamic observation of patients with stabilization of the tumor process, a case when, amid the stabilization, an increase in size of the tumor and the appearance of a vascular network was referred to as the continued growth of the tumor in the eye membranes. The case when tumor growth was recorded amid an atrophic chorioretinal focus (complete regression) was considered a relapse. The appearance of distant metastases in other organs was considered as the progression of the disease.

The SLE indicator was used to calculate the survival rate.

Таблица 1
Распределение опухолей в группах с учетом локализации опухоли на глазном дне
Table 1
The distribution of tumors in groups, taking into account the localization of the tumor in the fundus

Локализация Localization	Группа I Group I n=40		Группа II Group II n=51		Группа III Group III n=21	
	Абсолютное число Absolute number	%	Абсолютное число Absolute number	%	Абсолютное число Absolute number	%
Прилежит к диску зрительного нерва Adjacent to the optic nerve	3	7,5	2	3,9	1	4,8
Менее 3 мм (задний полюс) к диску зрительного нерва Less than 3 mm (posterior pole) to the optic disc	18	45,0	28	54,9	16	76,2
Более 3 мм от диска зрительного нерва More than 3 mm from the optic disc	5	12,5	5	9,8	2	9,5
Прилежит к макуле менее 3 мм Adjacent to the macula less than 3 mm	10	25,0	9	17,6	2	9,5
Отстоит от макулы более 3 мм More than 3 mm away from the macula	2	5,0	2	3,9	0	0
Периферия Periphery	2	5,0	5	9,8	0	0

Results

After PDT in the general group (n=112), complete tumor resorption was registered in the case of 29 (25.9%) patients, and stabilization of the tumor process was registered in the case of 83 (74.1%) patients with follow-up periods from 2.5 months to 3 years. Continued growth and relapses amid the stabilization and continued tumor growth were registered in the case of 34 patients: 25 (22.3%) men and 9 (8.0%) women, respectively. 5 (4.5%) enucleations were carried out to treat patients with continued tumor growth and recurrence. The eyeball was preserved after treatment of relapses and continued growth in the case of 29 (85.3%) patients. It was possible to preserve the eyeball in the general group of 107 (95.5%) patients. SLE constituted 100%, 3-year and 5-year 95,8±2,4%, 93,7±3,1%, accordingly.

In group I, 10 (25.0%) complete tumor resorption with the formation of a chorioretinal atrophic focus and 30 (75.0%) stabilization of the tumor process were registered. During the dynamic observation, continued growth amid recorded stabilization was detected in the case of 1 (2.5%) patient 5 months after treatment, the patient underwent brachytherapy (BT). 2 (5.0%) relapses were detected after 1 year and 1.5 years, patients underwent BT. In the case of 5 (12.5%) patients with synchronous and metachronous disease, there was no continued growth and recurrence of the tumor. All patients are alive. Complications and progression of the disease have not been established.

In group II, 15 (29.4%) patients had complete tumor resorption, 36 (70.6%) had stabilization of the tumor process. Continued growth amid recorded stabilization was noted in the case of 14 (27.5%) patients during the dynamic follow-up period from 2 to 8 months. 11 (21.6%) of these patients underwent BT, 1 (2.0%) patient underwent combined laser treatment, including PDT and TTT courses. 2 (4.0%) patients underwent enucleation. Relapse was registered in the case of 7 (13.7%) patients during the follow-up period from 8 months up to 2 years. Five (9.8%) patients were cured by PDT courses, and 2 (4.0%) patients underwent combined laser treatment using PDT and TTT. No complications of treatment have been registered. Among 7 (13.7%) patients with synchronous and metachronous disease, 4 patients had continued growth (3 patients) of the tumor and one patient had relapse of the disease. 2 patients were diagnosed with the progressed disease in the liver (the patient died) and bones (the patient is alive) in the first and second year after diagnosis and treatment.

In group III, 4 (19.0%) patients were diagnosed with complete tumor resorption, 17 (81.0%) – with stabilization of the tumor process. Continued growth amid recorded stabilization was registered in the case of 10 (46.7%) patients during dynamic follow-up period from 3 months up to 1.2 years. 8 of these patients underwent combined treatment, including TTT and BT courses. 2 patients underwent laser therapy courses, including TTT and PDT. Subsequently, 2 patients underwent enucleation. 3 (14.3%) patients had developed complications: secondary retinal detachment, hemorrhage into the eye membranes, opticoretinopathy after 11 months of continued growth and the second course of BT. 2 patients from 7 (33.3%) with synchronous and metachronous disease had continued growth. One patient died from the progression of the disease in the liver two years after the registration of continued tumor growth in the membranes of the eye.

Tumor resorption is slow during dynamic observation after PDT, so the proportion of stabilization of the tumor process is 3 times higher than full tumor resorption ($p<0.05$). The localization of the tumor in the vascular membrane of the eye (central zone, equator, periphery) does not affect the result of PDT ($p>0.05$).

Table 2 shows the indicators of SLE by study groups.

Таблица 2
Показатели скорректированной кумулятивной выживаемости по группам исследования
Table 2
Indicators of adjusted cumulative survival by study groups

Скорректированная кумулятивная выживаемость Adjusted cumulative survival	Группа I Group I	Группа II Group II	Группа III Group III
1-летняя (%) 1-year old (%)	100,0	100,0	100,0
3-летняя (%) 3-year old (%)	100,0	94,9±3,6	93,3±5,4
5-летняя (%) 5-year old (%)	100,0	93,3±6,4	93,3±5,4

The result of the antitumor efficacy of PDT did not depend on the localization of the tumor ($p>0.05$).

Cumulative indicators of 1-year SLE in the general group were 100%. 5-year SLE in the first group consisted 100%. In the second group, where tumors were larger, 3-year and 5-year survival was 94.9±3.6% and 93.3±6.4%, respectively. 3-year and 5-year SLE was 93.3±5.4% in the group where the observed tumors after PDT were the largest.

Discussion

The mechanism of PDT action is based on the selective accumulation of photosensitizing medicaments inserted into the body in cells with increased mitotic activity (in tumor cells, endothelium of newly formed vessels, etc.). Subsequent irradiation of a weakly pigmented tumor with light with a wavelength corresponding to the maximum of the absorption band of the injected photosensitizer induces photochemical reactions in sensitized cells and tissues with the release of singlet oxygen and free radicals – highly active biological oxidants, which leads to phototoxic damage to pathologically altered cells [10, 11]. The selectivity of the action determines the undoubted advantages of PDT for use in ophthalmology. This mechanism of action on a weakly pigmented tumor amid antitumor exposure allows to obtain the least destructive effect on adjacent structures, which is attractive for achieving the preservation of visual acuity associated with the absence of the development of vasculitis and subsequent opticoretinopathy.

However, sporadic studies describing the results of treatment of small tumors are devoted to the use of PDT in CM [12, 13]. One of the main reasons hindering the development of this direction in the world is the lack of photosensitizers with the necessary photophysical and pharmacokinetic properties. The production of photolon in the Republic of Belarus, which has high photodynamic activity with low skin phototoxicity and rapid elimination from the body, as well as the improvement of laser technology, bring new perspectives for a wider integration of the PDT method into ophthalmological practice.

Reviewing scientific and medical literature sources, the use of verteporphyrin and an insignificant number of patients in groups (from 8 patients in the smallest group up to 38 patients in the largest one) attracts attention [4-23]. It indicates a small world experience of PDT use in the treatment of CM. Short follow–up periods after the treatment are presented: the shortest ones are 15, 27 and 31 months, respectively, and the longest follow–up period is 3.5 and 5 years in one observation. The sizes of tumors that can be overthrown by PDT vary greatly, according to the data presented by the authors. This is due to the used PDT protocols (from 1 to many sessions before the expected result is obtained). According to some publications, PDT is most effective in melanomas with a tumor thickness of <4 mm [4], in other publications, the average thickness of the tumor subjected to PDT was 2.7 mm [25]. Some authors conduct PDT in tumors reaching a height of up to 4.4 mm [25] and even up to 5.7 mm [23]. In all unsuccessful cases, the tumors were 100% pigmented, there was a de novo CM, not transformed nevi, the radial nature of tumor growth, and not an increase in its thickness [21].

Tumor pigmentation plays a significant role in obtaining a positive result from PDT. It is known that the pigment shields the cells and vessels of the tumor. Hence, highly pigmented tumors do not react well to PDT. We do not use PDT for pigmented tumors in our treatment experience. However, some authors, on the contrary, note a positive effect in the treatment of pigmented CM [19, 20, 21]. One publication clearly shows that it was the well-pigmented part of the tumor that did not respond to PDT in the case of a mixed form of pigmentation [23]. The PDT method is most effective in low-pigmented forms of CM. The advantage of the PDT method is a decrease in the level of subretinal fluid after treatment, the presence of only isolated cases of fluid increase are recorded [19, 21, 22]. The amount of subretinal fluid decreased after treatment ($p<0.001$), vision did not deteriorate ($p=0.11$) and even improved in the case of the patients with a subfoveal tumor location ($p=0.018$) [21]. It has also been shown that photodynamic therapy does not significantly affect visual acuity [22, 23, 25].

According to literature data, 73.4% of patients recovered 1 month after PDT [8], 80% of cured patients 15 months after PDT [20], 62% of cured patients 27 months later [19], 80% of cured patients 31 months later [4], after PDT performed using the verteporfin photosensitizer, with a follow–up period of 5 years of cured patients – 67% [22]. All authors claim that PDT does not cause serious complications that cause visual acuity decreases. Some authors describe cases when the amount of subretinal fluid decreases up to complete resorption after the use of PDT [19, 21, 22]. Among the described complications, the authors note local atrophy of the retinal pigment epithelium at the treatment site in 25% of the eyes without affecting the function of the macular or optic nerve [22]. The cases of 2 patients with developed scleritis requiring a short course of systemic steroids were described [25].

Conclusion

The main criterion for choosing the method concerning the treatment of patients with weakly pigmented forms of melanoma of the vascular membrane of the eye is the size of the tumor. The best results were

obtained with a tumor thickness of up to 1.7 mm and a basal diameter of 10.3 mm, (25.0% complete tumor resorption and 75.0% stabilization of the tumor process with an adjusted 5-year survival rate of 100%). Photodynamic therapy shows high rates of adjusted 1-year cumulative survival – 100%, 3-year and 5-year – 95.8± 2.4% and 93.7± 3.1%, respectively. Photodynamic therapy being one of the methods of laser therapy can be used to preserve the eyeball and visual functions in the treatment of patients with relapse and continued growth of low-pigmented forms of melanoma of the vascular membrane of the eye.

REFERENCES

1. Naumenko L.V., Zhiljaeva E.P., Evmenenko A.A. Analiz statisticheskih pokazatelej zabolevaemosti melanomoj sosudistoj obolochki glaza v Respublike Belarus' za period 1997–2016 gg. [Analysis of statistical indicators of the incidence of a melanoma of the vascular membrane of the eye in the Republic of Belarus for the period 1997–2016], *Onkologicheskij zhurnal,* 2018, vol. 12, no. 3–4, pp. 21–28.
2. Dalidovich A.A., Marchenko L.N., Fedulov A.S. i dr. Fotodinamicheskaja terapija fotolonom miopaticheskoj makulopatii [Photodynamic therapy with a photolone of myopathic maculopathy], Minsk, *Paradoks*, 2012. – 224 p.
3. Filonenko E.V. Clinical implementation and scientific development of photodynamic therapy in Russia in 2010-2020. *Biomedical Photonics*, 2021, Vol. 10(4), pp. 4-22. doi: 10.24931/2413-9432-2021-9-4-4-22
4. Cerman E, Çekiç O. Clinical use of photodynamic therapy in ocular tumors, *Surv Ophthalmol*, 2015, vol. 60(6), pp. 557–574. doi: 10.1016/j.survophthal.2015.05.004.
5. Canal-Fontcuberta I., Salomão D.R., Robertson D. et al. Clinical and histopathologic findings after photodynamic therapy of choroidal melanoma, *Retina*, 2012., vol. 32(5), pp. 942–948. doi: 10.1097/IAE.0b013e31825097c1.
6. Rundle P. Photodynamic therapy for eye cancer, *Biomedicines*, 2017, vol. 5(4), pp. 69–75. doi: 10.3390/biomedicines5040069.
7. Blasi M.A., Pagliara M.M., Lanza A. et al. Photodynamic therapy in ocular oncology, *Biomedicines,* 2018, vol. 6(1), pp. 17–22. doi: 10.3390/biomedicines6010017.
8. Blasi M.A., Laguardia M., Tagliaferri L. et al. Brachytherapy alone or with neoadjuvant photodynamic therapy for amelanotic choroidal melanoma: functional outcomes and local tumor control, *Retina,* 2016, vol. 36(11), pp. 2205–2212. doi: 10.1097/IAE.0000000000001048.
9. Kawczyk-Krupka A., Bugaj A.M., Latos W. et al. Photodynamic therapy in treatment of cutaneous and choroidal melanoma, *Photodiagnosis Photodyn Ther,* 2013, vol. 10(4), pp. 503–509. doi: 10.1016/j.pdpdt.2013.05.006.
10. Jori G. Photosensitized processes in vivo: proposed phototherapeutic applications, *Photochem. Photobiol*, 1990, vol. 52(2), pp. 439–443. doi: 10.1111/j.1751-1097.1990.tb04201.x.
11. Kessel D. Pharmacokinetics of N-aspartylchlorin e6 in cancer patients, *J. Photochem. Photobiol*, 1997, vol. 39(1), pp. 81–83. doi: 10.1016/s1011-1344(96)00009-7.
12. Naumenko L.V. Avastin i fotodinamicheskaja terapija s fotolonom v izuchenii protivoopuholevoj jeffektivnosti v jeksperimente na zhivotnyh [Avastin and photodynamic therapy with a photolone in the study of antitumor efficiency in an animal experiment], *Onkologicheskij zhurnal,* 2012, vol. 6, no. 4, pp. 30–37.
13. Belyj Ju.A., Tereshhenko A.V., Volodin P.L. i dr. Transpupilljarnaja fotodinamicheskaja terapija melanomy horioidei srednih razmerov s preparatom «Fotoditazin» (klinicheskij sluchaj) [Transpupyllar photodynamic therapy of medium -sized choroids with the drug "Photo-Divine" (clinical case)], *Refrakcionnaja hirurgija i oftal'mologija,* 2008, vol.8, no. 1, pp. 22–26.
14. Belyj Ju.A., Tereshhenko A.V., Volodin P.L. i dr. Jeksperimental'nye rezul'taty fotodinamicheskoj terapii v oftal'mologii s

ЛИТЕРАТУРА

1. Науменко Л.В., Жиляева Е.П., Евмененко А.А. Анализ статистических показателей заболеваемости меланомой сосудистой оболочки глаза в Республике Беларусь за период 1997–2016 гг. // Онкологический журнал. – 2018. – Т. 12, № 3–4. – С. 21–28.
2. Далидович А.А., Марченко Л.Н., Федулов А.С. и др. Фотодинамическая терапия фотолоном миопатической макулопатии // Минск: Парадокс. – 2012. – 224 с.
3. Филоненко Е.В. Клиническое внедрение и научное развитие фотодинамической терапии в России в 2010-2020 гг. // Biomedical Photonics. – 2021. – Т. 10, № 4. – С. 4–22. doi: 10.24931/2413-9432-2021-9-4-4-22
4. Cerman E, Çekiç O. Clinical use of photodynamic therapy in ocular tumors // Surv Ophthalmol. – 2015. – Vol. 60(6). – P. 557–574. doi: 10.1016/j.survophthal.2015.05.004.
5. Canal-Fontcuberta I., Salomão D.R., Robertson D. et al. Clinical and histopathologic findings after photodynamic therapy of choroidal melanoma // Retina. – 2012. – Vol. 32(5). – P. 942–948. doi: 10.1097/IAE.0b013e31825097c1.
6. Rundle P. Photodynamic therapy for eye cancer // Biomedicines. – 2017. – Vol. 5(4). – P. 69–75. doi: 10.3390/biomedicines5040069.
7. Blasi M.A., Pagliara M.M., Lanza A. et al. Photodynamic therapy in ocular oncology // Biomedicines. – 2018. – Vol. 6(1). – P. 17–22. doi: 0.3390/biomedicines6010017.
8. Blasi M.A., Laguardia M., Tagliaferri L. et al. Brachytherapy alone or with neoadjuvant photodynamic therapy for amelanotic choroidal melanoma: functional outcomes and local tumor control // Retina. – 2016. – Vol. 36(11). – P. 2205–2212. doi: 10.1097/IAE.0000000000001048.
9. Kawczyk-Krupka A., Bugaj A.M., Latos W. et al. Photodynamic therapy in treatment of cutaneous and choroidal melanoma // Photodiagnosis Photodyn Ther. – 2013. – Vol. 10(4). – P. 503–509. doi: 10.1016/j.pdpdt.2013.05.006.
10. Jori G. Photosensitized processes in vivo: proposed phototherapeutic applications. // Photochem. Photobiol. – 1990. – Vol. 52(2). – P. 439–443. doi: 10.1111/j.1751-1097.1990.tb04201.x.
11. Kessel D. Pharmacokinetics of N-aspartylchlorin e6 in cancer patients // J. Photochem. Photobiol. – 1997. – Vol. 39(1). – P. 81–83. doi: 10.1016/s1011-1344(96)00009-7.
12. Науменко Л.В. Авастин и фотодинамическая терапия с фотолоном в изучении противоопухолевой эффективности в эксперименте на животных // Онкологический журнал. – 2012. – Т. 6, № 4. – С. 30–37.
13. Белый Ю.А., Терещенко А.В., Володин П.Л. и др. Транспупиллярная фотодинамическая терапия меланомы хориоидеи средних размеров с препаратом «Фотодитазин» (клинический случай) // Рефракционная хирургия и офтальмология. – 2008. – Т. 8, № 1. – С. 22–26.
14. Белый Ю.А., Терещенко А.В., Володин П.Л. и др. Экспериментальные результаты фотодинамической терапии в офтальмологии с использованием препаратов хлоринового ряда // Рефракционная хирургия и офтальмология. – 2007. – Т. 7, № 1. – С. 27–34.

ispol'zovaniem preparatov hlorinovogo rjada [Experimental results of photodynamic therapy in ophthalmology using chlorin preparations], *Refrakcionnaja hirurgija i oftal'mologija,* 2007, vol. 8, no.1, pp. 27–34.

15. Naumenko L.V., Cerkovskij D.A., Shishlo L.M. Vlijanie kombinirovannogo vozdejstvija fotodinamicheskoj terapii s fotolonom, lazernoj termoterapii, brahiterapii i targetnoj himioterapii na syvorotochnye urovni VEGF, NSE i s100 u jeksperimental'nyh zhivotnyh [The influence of the combined effects of photodynamic therapy with photolone, laser thermotherapy, brachytherapy and targeted chemotherapy for serum levels of VEGF, NSE and S100 in experimental animals], *Onkologicheskij zhurnal,* 2014, vol. 8, no. 1, pp. 46–50.
16. Belyj Ju.A., Tereshhenko A.V., Volodin P.L. i dr. Pervye jeksperimental'nye rezul'taty fotodinamicheskoj terapii v oftal'mologii s ispol'zovaniem otechestvennogo preparata «Fotoditazin» [The first experimental results of photodynamic therapy in ophthalmology using the domestic drug "Photoditazin"], *Vestnik Orenburgskogo gos. un-ta,* 2004, no. 12, pp. 182–185.
17. Schlötzer-Schrehardt U., Viestenz A., Naumann G.O. et al. Dose-related structural effects of photodynamic therapy on choroidal and retinal structures of human eyes, *Graefes Arch Clin Exp Ophthalmol*, 2002, vol. 240(9), pp. 748–757. doi: 10.1007/s00417-002-0517-4.
18. Baldea I., Filip A.G. Photodynamic therapy in melanoma – an update, *J Physiol Pharmacol*, 2012, vol. 63(2), pp. 109–118.
19. Fabian I.D., Stacey A.W., Harby L.A. et al. Primary photodynamic therapy with verteporfin for pigmented posterior pole cT1a choroidalmelanoma: a 3-year retrospective analysis, *Br J Ophthalmol*, 2018, vol. 102(12), pp. 1705–1710. doi: 10.1136/bjophthalmol-2017-311747.
20. Jmor F., Hussain R.N., Damato B.E. et al. Photodynamic therapy as initial treatment for small choroidal melanomas, *Photodiagnosis Photodyn Ther,* 2017, vol. 20, pp. 175–181. doi: 10.1016/j.pdpdt.2017.10.018.
21. Fabian I.D., Stacey A.W., Papastefanou V. et al. Primary photodynamic therapy with verteporfin for small pigmented posterior pole choroidal melanoma, *Eye (Lond)*, 2017, vol. 31(4), pp. 519–528. doi: 10.1038/eye.2017.22.
22. Turkoglu E.B., Pointdujour-Lim R., Mashayekhi A. et al. Photodynamic therapy as primary treatment for small choroidal melanoma, *Retina*, 2019, vol. 39(7), pp. 1319–1325. doi: 10.1097/IAE.0000000000002169.
23. Campbell W.G., Pejnovic T.M. Treatment of amelanotic choroidal melanoma with photodynamic therapy, *Retina*, 2012, vol. 32(7), pp. 1356–1362. doi: 10.1097/IAE.10.1097/IAE.0b013e31822c28ec.
24. O'Day R.F., Pejnovic T.M., Isaacs T. et al. Australian and New Zealand study of photodynamic therapy in choroidal amelanotic melanoma, *Retina*, 2020, vol. 40(5), pp. 972–976. doi: 10.1097/IAE.0000000000002520.
25. Rundle P. Treatment of posterior uveal melanoma with multi-dose photodynamic therapy, *Br J Ophthalmol*, 2014, vol. 98(4), pp. 494–497. doi: 10.1136/bjophthalmol-2013-304432.

15. Науменко Л.В., Церковский Д.А., Шишло Л.М. Влияние комбинированного воздействия фотодинамической терапии с фотолоном, лазерной термотерапии, брахитерапии и таргетной химиотерапии на сывороточные уровни VEGF, NSE и s100 у экспериментальных животных // Онкологический журнал. – 2014. – Т. 8, № 1. – С. 46–50.
16. Белый Ю.А., Терещенко А.В., Володин П.Л. и др. Первые экспериментальные результаты фотодинамической терапии в офтальмологии с использованием отечественного препарата «Фотодитазин». – Вестник Оренбургского гос. ун-та. – 2004. – № 12. – С. 182–185.
17. Schlötzer-Schrehardt U., Viestenz A., Naumann G.O. et al. Dose-related structural effects of photodynamic therapy on choroidal and retinal structures of human eyes // Graefes Arch Clin Exp Ophthalmol. – 2002. – Vol. 240(9). – P. 748–757. doi: 10.1007/s00417-002-0517-4.
18. Baldea I., Filip A.G. Photodynamic therapy in melanoma – an update // J Physiol Pharmacol. – 2012. – Vol. 63(2). – P. 109–118.
19. Fabian I.D., Stacey A.W., Harby L.A. et al. Primary photodynamic therapy with verteporfin for pigmented posterior pole cT1a choroidalmelanoma: a 3-year retrospective analysis // Br J Ophthalmol. – 2018. – Vol. 102(12). – P. 1705–1710. doi: 10.1136/bjophthalmol-2017-311747.
20. Jmor F., Hussain R.N., Damato B.E. et al. Photodynamic therapy as initial treatment for small choroidal melanomas //Photodiagnosis Photodyn Ther. – 2017. – Vol. 20. – P. 175–181. doi: 10.1016/j.pdpdt.2017.10.018.
21. Fabian I.D., Stacey A.W., Papastefanou V. et al. Primary photodynamic therapy with verteporfin for small pigmented posterior pole choroidal melanoma // Eye (Lond). – 2017. – Vol. 31(4). – P. 519–528. doi: 10.1038/eye.2017.22.
22. Turkoglu E.B., Pointdujour-Lim R., Mashayekhi A. et al. Photodynamic therapy as primary treatment for small choroidal melanoma // Retina. – 2019. – Vol. 39(7). – P.1319–1325. doi: 10.1097/IAE.0000000000002169.
23. Campbell W.G., Pejnovic T.M. Treatment of amelanotic choroidal melanoma with photodynamic therapy // Retina. – 2012. – Vol. 32(7). – P. 1356–1362. doi: 10.1097/IAE.10.1097/IAE.0b013e31822c28ec.
24. O'Day R.F., Pejnovic T.M., Isaacs T. et al. Australian and New Zealand study of photodynamic therapy in choroidal amelanotic melanoma // Retina. – 2020. – Vol. 40(5). – P. 972–976. doi: 10.1097/IAE.0000000000002520.
25. Rundle P. Treatment of posterior uveal melanoma with multi-dose photodynamic therapy // Br J Ophthalmol. – 2014. – Vol. 98(4). – P. 494–497. doi: 10.1136/bjophthalmol-2013-304432.

REVIEWS OF LITERATURE

PHOTODYNAMIC THERAPY IN THE TREATMENT OF EXTRAMAMMARY PAGET'S DISEASE

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

Abstract

Extramammary Paget's disease (EMPD) is a rare tumor that predominantly affects the skin containing apocrine glands. Due to insufficient data on the effectiveness of different methods, there is no single therapeutic approach to the treatment of patients with EMPD and their subsequent management. The use of surgical methods, laser therapy and local cytotoxic drugs has a number of limitations. The advantages of using photodynamic therapy (PDT) in EMPD are the absence of systemic toxicity, non-invasiveness, selectivity of action, the absence of carcinogenic potential, the possibility of conducting several courses of treatment, and good cosmetic results. In our review, we analyzed those published in 2000-2022 data on the results of PDT treatment of 114 patients with EMPD. As a result of treatment, complete regression of tumor foci was achieved in 40% of patients. Most authors note that PDT is more effective for small areas (up to 4 cm^2).

Key words: photodynamic therapy, extramammary Paget's disease, 5-aminolevulinic acid, photofrin, 5-aminolevulinic acid methyl ester.

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

For citations: Filonenko E.V., Ivanova-Radkevich V.I. Photodynamic therapy in the treatment of extramammary Paget disease, *Biomedical Photonics*, 2022, vol. 11, no. 3, pp. 24–34. doi: 10.24931/2413–9432–2022–11-3-24–34.

ФОТОДИНАМИЧЕСКАЯ ТЕРАПИЯ В ЛЕЧЕНИИ ЭКСТРАМАММАРНОГО РАКА ПЕДЖЕТА

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

Резюме

Экстрамаммарный рак Педжета (ЭМРП) – редко встречающаяся опухоль, которая преимущественно поражает кожу, содержащую апокринные железы. Из-за недостаточного количества данных по эффективности разных методов отсутствует единый терапевтический подход к лечению пациентов с ЭМРП и их последующего ведения. Применение хирургических методов, лазерной терапии и местных цитостатических препаратов имеет ряд ограничений. Преимуществами использования при ЭМРП фотодинамической терапии (ФДТ) являются отсутствие системной токсичности, неинвазивность, избирательность действия, отсутствие канцерогенного потенциала, возможность проведения нескольких курсов лечения и хорошие косметические результаты. В нашем обзоре проанализированы опубликованные в 2000-2022 гг. данные о результатах лечения методом ФДТ 114 пациентов с ЭМРП. В результате лечения полная регрессия опухолевых очагов была достигнута у 40% пациентов. Большинство авторов отмечают, что ФДТ эффективнее при очагах небольшой площади (до 4 $см^2$).

Ключевые слова: фотодинамическая терапия, экстрамаммарный рак Педжета, 5-аминолевулиновая кислота, фотофрин, метиловый эфир 5-аминолевулиновой кислоты.

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

Для цитирования: Филоненко Е.В., Иванова-Радкевич В.И. Фотодинамическая терапия в лечении экстрамаммарного рака Педжета // Biomedical Photonics. – 2022. – Т. 11, № 3. – С. 24–34. doi: 10.24931/2413–9432–2022–11-3-24–34.

Epidemiology

Extramammary Paget's disease (EMPD) is a rare, slow–growing intraepithelial adenocarcinoma that predominantly affects the skin containing apocrine glands, such as the perianal, genital, and axillary areas of the body. Elderly people are more often diagnosed with EMPD than the others, with such disease characteristics as slow development and progression [1-3]. Women tend to be exposed to EMPD more than men (3-4,5:1) [4]. In the case of ineffective treatment, the disease progresses into invasive and metastatic forms.

The frequency and prevalence of EMPD are not precisely determined, although the disease is generally considered to be rare. Most of the notes on EMPD in the dermatological literature over the past decades were reports about individual clinical observations. Due to the rare occurrence of EMPD, conducting full-fledged prospective multicenter clinical trials is difficult. Hence, there are no reports of such studies in the literature [5]. On average, the incidence of EMPD is estimated as only 0.12 per 100,000 people and constitute 21% of primary cases of scrotal cancer, 1-2% of primary cases of vulvar cancer [2, 6]. At the same time, Paget's mammary cancer accounts for 0.7-4.3% of all cases of breast cancer [7]. EMPD accounts for only 6.5% of all forms of Paget's disease [8]. In Germany, the number of new cases of EMPD is estimated at the level of 20-126 cases per year [5], in the Netherlands – 17 cases per year [9].

Clinic

EMPD affects areas of the skin containing apocrine sweat glands. Predisposition areas include the anogenital region and, less often, the armpits. A study conducted by J.J. Chanda et al. with the participation of 197 patients with EMPD showed that 128 (65%) patients had vulva affected, 29 (15%) patients had tumor in perianal areas, and another 27 (14%) patients had penis, scrotum or inguinal area affected [10]. In cases involving the vulva, in most cases, a tumor occurs on the labia majora [9].

Clinically, EMPD lesions are often manifested by infiltrative erythema with crusts and scales, sometimes resembling other skin diseases, such as eczema [1].

There are primary and secondary forms of EMPD. While primary EMPD occurs as an intraepithelial neoplasm of the epidermis, secondary EMPD develops as a result of the epidermotropic or direct spread of malignant cells from the main primary focus of the malignant neoplasm such as, usually, the lower gastrointestinal tract or urinary tract. As the treatment strategy and prognosis for primary and secondary EMPD differ, an accurate diagnosis based on a detailed histopathological assessment of a variety of immunohistochemical markers is required. In most cases, EMPD is diagnosed as carcinoma in situ, which usually demonstrates a slow progression of the disease. However, once Paget cells penetrate deep into the dermis, the risk of developing metastases in regional lymph nodes increases [1, 3].

Despite its slow progression, EMPD does not respond well to therapy. The overall 10-year survival rate for EMPD is about 60% after diagnosis [11].

The clinical manifestations of EMPD can vary significantly depending on the area and localization of the affected area and the duration of the disease. The primary lesion is usually represented by a red spot with sharp borders. Spots with depigmentation or hyperpigmentation may appear. The color can vary from pink, light red or dark red to red brown. In particular, larger lesions may have a mixed color. Larger, more common lesions may be irregularly shaped with poorly defined borders. The centrifugal nature of growth leads to the formation of uneven boundaries, the appearance of which is considered to be a characteristic feature of EMPD. The surface of the lesion may have a rough lamellar plaque depending on the affected area [5].

Diagnostics

Due to the similarity of the clinical picture with many benign conditions, the diagnosis of EMPD is often postponed for years, and only every fifth patient is correctly diagnosed at the first appointment [12]. More often, EMPD manifests itself in the form of well-defined or poorly defined erythematous and scaly plaques, which can become crusted, ulcerate, or acquire pigmentation [2]. But at an early stage, the most common symptoms are itching, rash or erythema, which usually do not cause any special suspicion of malignant etiology. Initial differential diagnoses are contact dermatitis, seborrheic eczema, and fungal infections. Patients with EMPD are often treated for the mentioned above diseases for several years with the use of the conservative treatment such as local emollients or corticosteroids, antifungal creams or other oral treatments. And only after a long period of unsuccessful treatment, an additional diagnosis is carried out. As a result, the correct diagnosis is postponed for several years [2].

The biopsy of the lesion helps to establish the final diagnosis. Characteristic signs of EMPD are a thickened epidermis with papillomatosis, an increase in interpapillary furrows, hyperkeratosis or parakeratosis on the surface, and characteristic Paget cells with transparent abundant cytoplasm. The cytoplasm of these cells is positive for Schiff acid staining and

resistant to diastasis, which indicates the presence of neutral polysaccharides and confirms the glandular origin of the cells. Markers of the glandular epithelium are also a positive reaction to staining with antibodies to carcinoembryonic antigen (CEA), low molecular weight cytokeratin (Cam 5.2), and cytokeratin 7 (CK7). Histological examination of the EMPD focus usually reveals epidermal infiltration of Paget cells, which look like large round cells with abundant pale pink cytoplasm surrounding hypochromatic nuclei, sometimes with a protruding nucleolus [13, 14].

Treatment

Due to the rare occurrence of EMPD, there are disagreements regarding therapeutic approaches to the treatment of patients with EMPD and their subsequent management. Currently, the recommendations for the EMPD treatment are based only on the published results of several series of clinical observations of individual institutions and small reports on individual clinical observations, with many different therapy options. Hence, it is difficult to compare and evaluate their effectiveness [2].

Several invasive and non-invasive EMPD treatment options are used with limited comparative data for the EMPD treatment.

Surgical treatment

Although there are no established guidelines for the EMPD treatment, surgical resection is the generally accepted standard of practice. In particular, wide local excision with edges from 1 to 5 cm is widely used, but its use may be associated with a high frequency of relapses. In several studies, it has been demonstrated that Mohs micrographic surgery provides high efficiency of treatment with a lower probability of relapses [2, 3, 15]. In case of Mohs micrographic surgery use, 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. Mohs micrographic surgery provides an intraoperative microscopic assessment of 100% of the lesion edges, which makes it possible to remove only the affected tissue and reduce the recurrence rate [16].

The problem with the use of Mohs micrographic surgery, as well as any other option for surgical treatment of EMPD, is that most cases of EMPD are represented by extensive lesions of a significant area. In addition, the disease primarily affects the elderly people. Not all of whom can undergo surgery. The necessary procedures in the surgical treatment of anogenital diseases (for example, vulvectomy, installation of a skin-muscle flap or artificial anus) often lead to a significant decrease in the quality of life [5]. In addition, many researchers note a rather high risk of recurrence after surgical treatment of EMPD. Thus, according to J.D. Zollo et al. [17], the average recurrence rate after surgical treatment for all EMPD localities is 35-44%. Hence, there is considerable interest in less invasive therapeutic approaches due to the potential slow progression of the disease and limitations in the use of surgical methods of treatment [15].

Local therapy

One of the alternatives to surgical intervention is the use of local cytostatic and other medications. In the studies of H.F. Haberman et al. [18] and Kawatsu T. and co-author. [19], it was shown that the use of 5-fluorouracil in the treatment of the genital form of EMPD makes it possible to eliminate the clinical manifestations of the disease. At the same time, the results of the biopsy showed the preservation of pathological cells, which indicates a low effectiveness of treatment. W.G. Watring et al. [20] reported a complete therapeutic response to topical bleomycin treatment in the case of 4 out of 7 patients with recurrent vulvar EMPD. One of the 4 patients had a relapse of the disease after 30 months, which required repeated treatment.

Another medicine for local therapy in EMPD treatment is imiquimod (an immunomodulator and modifier of the immune response, which enhances both innate and acquired immunity by stimulating the production of cytokines such as interferon-α and tumor necrosis factor-α). These cytokines, in turn, activate the antitumor immune system, increasing the death and destruction of tumor cells [2]. According to H. Machida et al. [21], the therapeutic efficacy of imiquimod in the treatment of EMPD reaches 52-80% with a recurrence rate of 19%.

Laser therapy

The advantages of using CO_2 and Nd:YAG lasers for the treatment of EMPD include non-invasiveness and a reduction in the duration of hospitalization compared to surgical intervention. However, the lack of histological data for analysis, postoperative pain and anesthesia requirements reduce the therapeutic value of this treatment method [2]. Some researchers note a high recurrence rate (up to 67-100%) in the case of using this method in the treatment of multifocal, extensive, and invasive lesions of EMPD [22, 23].

Photodynamic therapy

Photodynamic therapy (PDT) is a minimally invasive method of antitumor therapy, it is a procedure involving local or systemic administration of a photosensitizer that is selectively localized in tumor tissues. Subsequent activation of the medicine by a light source leads to the formation of reactive oxygen species, which causes the death of tumor cells. The advantages of PDT include low toxicity, the possibility of repeated courses without the development of resistance to treatment, preservation of organ function and good cosmetic results, as well as the possibility of use combined with other methods of treatment [3, 24-26]. The experience of using PDT in the treatment of EMPD estimates more than 30 years. However, most of the data in the literature on this topic are reports of single clinical observations with PDT in various modes, often using different photosensitizers treating one patient. In the available literature, there are no systematic reviews, meta-analyses of the effectiveness of the use of different PDT regimens in the treatment of EMPD or reports on the results of full-scale prospective clinical studies of the effectiveness of PDT in EMPD. These reasons significantly complicate the development of a single tactic for effective treatment of EMPD by PDT.

This review systematizes the results of EMPD treatment by PDT in mono-mode according to publications on pubmed.com for 2000-2022. We analyzed the results of PDT in the treatement of 114 patients with EMPD. The number of foci was indicated only in some publications, so it is not possible to estimate their total number. For PDT, ointments with 5-aminolevulenic acid (5-ALA) (29 patients) and 5-ALA methyl ether (67 patients) were most often used, photophrine (4 patients) and a hematoporphyrin derivative (11 patients) were also used. In the case of 3 patients, treatment was carried out using 5-ALA and photophrine (for different foci or sequentially for one focus).

There are no comparative data on the effectiveness of different photosensitizers for PDT in EMPD. Some authors point to a higher efficiency of PDT using photophrine compared to the local application of ointment based on 5-ALA. Thus, V. Madan et al. [27] reported the results of treatment of an 80-year-old patient with an extensive invasive focus of scrotal EMPD with an area of 100 cm^2, secondary to prostate adenocarcinoma by PDT. The patient had a relapse of the disease in 9 months after 5 courses of PDT with local application of 20% 5-ALK ointment. The patient underwent PDT with photofrin. Within 1 year after treatment, the patient was observed without registered relapse. Based on the obtained results, the authors of the study point to the high efficiency of PDT using photophrine. In the study of S. Shieh et al. [28] after PDT of 16 EMPD foci with 20% 5-ALA ointment, complete regression was obtained in 50% of cases (8 foci). One focus with partial regression of the tumor was additionally treated with PDT with photofrin at a dose of 1 mg/kg; the result was assessed as a complete regression with an effect duration of 71 months. Despite these isolated reports of the high efficacy of photophrine, a small amount of data does not allow us to draw reasonable conclusions about the benefits of its use for the treatment of EMPD.

Many patients treated with PDT had of a large area of EMPD foci. Thus, the lesion area in one of the patients exceeded 75 cm^2 in the study of X.L. Wang et al. [29], and the lesion area was about 100 cm^2 in the case of the patient described in the study V. Madan et al. [27]. Many authors note that the direct effect of PDT depends on the area of the treated focus. Thus, both patients in these studies had only partial regression of the tumor after PDT. In the work of R. Fontanelli et al. [30], it was noted that, during the treatment of patients with small formations (with a size of up to 5 cm^2), complete regression was obtained in the case of 50% of patients, with a size of 5-10 cm^2 foci – in 10% of cases, more than 10 cm^2 – in 0%.

The long-term effects of PDT also depend on the area of the EMPD focus. Q. Li et al. [31] note the relationship between the frequency of relapses and the area of the focus treated by PDT. In this study, both relapses registered within 12 months after PDT occurred in the case of the patients who had a focus area of more than 4 cm^2 (in total, 13 patients with a focus area of more than 4 cm^2 participated in the study). None of the patients with a focal area of less than 4 cm^2 (8 patients) had relapses for these periods. In the study of M. Magnano et al. [32], complete tumor regression was achieved after PDT treatment of a patient with a 15 cm^2 EMPD lesion. However, after 2 months, the patient had a relapse of the disease.

The main complication of PDT in patients with EMPD was severe pain during PDT. Q. Li et al. [31] report that 12 of the 16 patients who participated in the study could not withstand the acute pain caused by LED irradiation, and they were given anesthesia. The average intensity of pain on a scale from 1 to 10 was 5.4, including in case of perianal localization of the EMPD focus – 9.1, vulvar – 6.5, axillary 4.5 and scrotal – 3.2. At the same time, the study of F. Raspagliesi et al. [33] shows that pain was noted only in 1 out of 7 patients with vulvar localization of EMPD foci.

The table presents summary data on 15 publications dating from 2000 to 2022 (indexed in Pubmed) with the results of a study on the PDT effectiveness in a treatment of 114 patients with EMPD in total.

Таблица
Сводные данные результативности применения фотодинамической терапии у пациентов с ЭМРП
Table
Summary of the effectiveness of photodynamic therapy in patients with extramammary Paget's disease

Авторы Authors	Число пациентов/ количество очагов No. of lesions/ No. of patients	Локализация Localisation	Фотосенсибилизатор, доза Photosensitizer, dose	Световая доза, Дж/см² Light dose, J/cm²	Количество курсов ФДТ Number of PDT courses	Результат терапии Results of therapy	Рецидив Relapse	Срок наблюдения, мес Follow-up period, month
Runfola и соавт., 2000 [34] Runfola et al., 2000 [34]	1/7	Перианальная область Perianal area	Фотофрин 1 мг/кг Photofrin 1 mg/kg	210 Дж/см² 210 J/cm²	1 курс 1 course	Пациенты: ПР 100% (1/1) Patients: CR 100% (1/1)	Нет No	Не указано Not specified
Shieh и соавт., 2002 [28] Shieh et al., 2002 [28]	5/17	Пах, мошонка, половой, член, лобок Groin, scrotum, penis, pubis	5-АЛК 20% мазь, экспозиция 18-24 ч (17 очагов) Фотофрин 1 мг/кг (1 очаг) 5-ALA 20% ointment, exposure 18-24 hours (17 foci) Photofrin 1 mg/kg (1 focus)	100-400 Дж/см² 100-400 J/cm²	1-5 курсов (интервал 2 нед) 1-5 courses (interval of 2 weeks)	Очаги: ПР 53% (9/17) ЧР 18% (3/17) СТ % (29/17) Lesions: CR 53% (9/17) PR 18% (3/17) NC % (29/17)	После ФДТ с 5-АЛК и фотофрином – через 6 мес ПЭ, без рецидива 71 мес. После 5-АЛК – 3 рецидива через 9-10 мес, 13 без рецидива 6-29 мес. After PDT with 5-ALA and photofrin – after 6 months of PE, without relapse 71 months. After 5-ALA – 3 relapse after 9-10 months, 13 without relapse 6-29 months.	6-71 мес 6-71 months
Madan и соавт., 2005 [27] Madan et al., 2005 [27]	1/1	Мошонка Scrotum	5-АЛК 20% мазь, экспозиция 6 ч Фотофрин 1 мг/кг 5-ALA 20% ointment, exposure 6 hours Photofrin 1 mg/kg	100 Дж/см² 100 J/cm²	5 курсов ФДТ с 5-АЛК (интервал 1-9 мес), затем 1 курс ФДТ с фотофрином 5 courses of PDT with 5-ALA (interval of 1-9 months), then 1 course of PDT with photofrin	Пациенты: ПР 100% (1/1) Patients: CR 100% (1/1)	Рецидив через 9 мес после 5 курсов ФДТ с 5-АЛК. После ФДТ с фотофрином без рецидива 12 мес Relapse 9 months after 5 courses of PDT with 5-ALA. After PDT with photofrin without relapse 12 months	12 мес 12 months

Raspagliesi и соавт., 2006 [33] Raspagliesi et al., 2006 [33]	7/11	Вульва * у 2 пациентов также были поражения вне вульвы, у 3 – поражения большой площади (площадь не указана) Vulva * 2 patients also had lesions outside the vulva, 3 had lesions of a large area (area not specified)	Метиловый эфир 5-АЛК 20% мазь, экспозиция 3 ч 5-ALA methyl ester 20% ointment, exposure 3 hours	37 Дж/см^2 37 J/cm^2	2-3 курса (интервал 3 нед) 2-3 courses (interval of 3 weeks)	Очаги: ПР 73% (8/11) ЧР 9% (1/11) СТ 18% (2/11) * 2 ПР не подтверждены гистологически Lesions: CR 73% (8/11) PR 9% (1/11) NC 18% (2/11)	Нет No	1-5 мес 1-5 months
Wang и со-авт., 2008 [29] Wang et al., 2008 [29]	3/4	Половой член Penis	5-АЛК 10-20% раствор, экспозиция 3-5 ч 5-ALA 10-20% solution, exposure 3-5 h	80-100 Дж/см^2 80-100 J/cm^2	2 курса (интервал 2 нед) 2 courses (interval of 2 weeks)	Пациенты: ЧР 100% (3/3) Patients: PR 100% (3/3)	2 рецидива в течение 12 мес 2 relapses within 12 months	12 мес 12 months
Tanaka и соавт., 2009 [13] Tanaka et al., 2009 [13]	4/не указано 4/not stated	Вульва, мошонка, подмышечная впадина Vulva, scrotum, armpit	Метиловый эфир 5-АЛК 16% мазь, экспозиция 3 ч 5-ALA methyl ester 16% ointment, exposure 3 hours	37 Дж/см^2 37 J/cm^2	3-8 курсов (интервал не указан) 3-8 courses (interval is not mentioned)	Пациенты: ПР 25% (1/4) ЧР 75% (3/4) Patients: CR 25% (1/4) PR 75% (3/4)		6-12 мес 6-12 months
Li и соавт., 2010 [31] Li et al., 2010 [31]	17/21	Мошонка, подмышечная область, перианальная область, вульва Scrotum, axillary region, perianal region, vulva	5-АЛК 20% мазь, экспозиция 6 ч 5-ALA 20% ointment, exposure 6 hours	113 Дж/см^2 113 J/cm^2	3 курса (интервал 1 нед) 3 courses (1 week interval)	Очаги: ПР 52,4% (11/21) Lesions: CR 52,4% (11/21)	2 рецидива через 6-12 мес 2 relapses after 6-12 months	24 мес 24 months
Housel и соавт., 2010 [35] Housel et al., 2010 [35]	8/25	Мошонка, подмышечная область, перианальная область, лобок, ягодица, половой член Scrotum, axillary region, perianal region, pubis, buttock, penis	5-АЛК 20% мазь (16 очагов) Фотофрин 1-2 мг/кг (9 очагов) 3 пациента: фотофрин, 4 пациента: 5-АЛК, 1 пациент – 5-АЛК + фотофрин 5-ALA 20% ointment (16 foci) Photofrin 1-2 mg/kg (9 foci) 3 Patienst: Photofrin, 4 patients: 5-ALA, 1 patient-5-ALA + fotofrin	5-АЛК: 100-250 Дж/см^2 Фото-фрин: 215 Дж/см^2 5-ALA: 100-250 J/cm^2 Photo-frin: 215 J/cm^2	5-АЛК: 1-5 курсов Фотофрин: 1 курс 5-ALA: 1-5 courses Photofrin: 1 course	Очаги, пролеченные 5-АЛК: ПР 50% (8/16) ЧР 19% (3/16) СТ 31% (5/16) Очаги, пролеченные фотофрином: ПР 78% (7/9) ЧР 22% (2/9) Foci treated with 5-ALA: PR 50% (8/16) CR 19% (3/16) NC 31% (5/16) Foci treated with photo-frin: PR 78% (7/9) CR 22% (2/9)	3 рецидива через 9-10 мес 3 relapses after 9-10 months	3-96 мес 3-96 months

Kitagawa и соавт., 2011 [36] Kitagawa et al., 2011 [36]	1/1	Надлобковая зона Suprapubic zone	Метиловый эфир 5-АЛК 16% мазь, экспозиция 3 ч 5-ALA methyl ester 16% ointment, exposure 3 hours	37 Дж/см^2 37 J/cm^2	2 курса (интервал 1 нед) 2 courses (1 week interval)	Пациенты: ПР 100% (1/1) Patients: CR 100% (1/1)	Нет No	2 мес 2 months
Yousef и соавт., 2012 [37] Yousef et al., 2012 [37]	2/не указано 2/not stated	Вульва, лобок, мошонка Vulva, pubis, scrotum	Метиловый эфир 5-АЛК 16% мазь, экспозиция 3 ч 5-ALA methyl ester 16% ointment, exposure 3 hours	37 Дж/см^2 37 J/cm^2	3 и 9 курсов (интервал 2-4 нед) 3 and 9 courses (interval of 2-4 weeks)	Пациенты: ЧР 50% (1/2) СТ 50% (1/2) Patients: PR 50% (1/2) NC 50% (1/2)	Нет No	12 мес 12 months
Fontanelli и соавт., 2013 [30] Fontanelli et al., 2013 [30]	32/не указано 32/not stated	Вульва, перианальная область Vulva, perianal area	Метиловый эфир 5-АЛК 16% мазь, экспозиция 3 ч 5-ALA methyl ester 16% ointment, exposure 3 hours	37 Дж/см^2 37 J/cm^2	3 курса (интервал 3 нед) 3 courses (interval of 3 weeks)	После 3 курсов: Пациенты: ПР 9% (3/32) ЧР 78% (25/32) СТ 13% (4/32) After 3 courses: Patients: CR 9% (3/32) PR 78% (25/32) NC 13% (4/32)	Рецидивы у 3/3 пациентов с ПР через 6, 8 и 18 мес Relapses in 3/3 of patients with CR after 6, 8 and 18 months	6-18 мес после ПР 6-18 months after CR
Magnano и соавт., 2013 [32] Magnano et al., 2013 [32]	1/1	Вульва Vulva	Метиловый эфир 5-АЛК 16% мазь, экспозиция 3 ч 5-ALA methyl ester 16% ointment, exposure 3 hours	37 Дж/см^2 37 J/cm^2	3 курса (интервал 3 нед) После рецидива 3 курса (интервал 3 нед) 3 courses (interval of 3 weeks) After relapse of the 3rd course (interval of 3 weeks)	После 6 курсов ФДТ: Пациенты: ПР 100% (1/1) After 6 courses: Patients: CR 100% (1/1)	Рецидив через 2 мес после 3 курсов Relapse in 2 months after 3 courses	Не указано Not specified
Calzavara-Pinton и соавт., 2013 [38] Calzavara-Pinton et al., 2013 [38]	8/не указано 8/not stated		Метиловый эфир 5-АЛК 16% мазь, экспозиция 3-4 ч 5-ALA methyl ester 16% ointment, exposure 3-4 hours	37 Дж/см^2 37 J/cm^2	В среднем 2,4 курса (интервал 3 нед) On average, 2.4 courses (interval of 3 weeks)	Пациенты: ПР 13% (3/8) ЧР 50% (4/8) СТ 37% (1/8) Patients: CR 13% (3/8) PR 50% (4/8) NC 37% (1/8)	Не указано Not specified	8,5 мес 8,5 months

Rioli и соавт., 2018 [6] Rioli et al., 2018 [6]	13/не указано 13/not stated	Вульва Vulva	Метиловый эфир 5-АЛК 16% мазь, экспозиция 3 ч 5-ALA methyl ester 16% ointment, exposure 3 hours	37 Дж/см^2 37 J/cm^2	1-3 курса (интервал не указан) 1-3 courses (interval is not mentioned)	После 1 курса ФДТ: Пациенты: ПР 38% (2/13) ЧР 38% (5/13) СТ 16% (5/13) РО 8% (1/13) После 2 курса ФДТ: Пациенты: ПР 17% (1/6) СТ 66% (4/6) РО 17% (1/6) После 3 курса ФДТ: Пациенты: СТ 100% (2/2) After the 1st course of PDT: Patients: CR 38% (2/13) PR 38% (5/13) NC 16% (5/13) RO 8% (1/13) After the 2nd course of PDT: Patients: CR 17% (1/6) NC 66% (4/6) RO 17% (1/6) After the 3rd course of PDT: Patients: NC 100% (2/2)	7 рецидивов у 7/7 пациентов с ПР и ЧР после 1 курса ФДТ 7 relapses in 7/7 patients with CR and PR after 1 course of PDT	4-75 мес 4-75 months
Wang и соавт., 2022 [39] Wang et al., 2022 [39]	11/не указано 11/not stated	Вульва, половой член, мошонка, перианальная область Vulva, penis, scrotum, perianal area	Производное гематопорфирина 3-5 мг/кг Hematoporphyrin derivative 3-5 mg/kg	150-200 Дж/см^2 150-200 J/cm^2		Пациенты: ПР 91% (10/11) ЧР 9% (1/11) Patients: CR 91% (10/11) PR 9% (1/11)	2 рецидива у 2/10 пациентов с ПР через 12 мес 2 relapses in the case of 2/10 patients with CR after 12 months	12-27 мес 12-27 months

Примечания: ПР – полная регрессия, ЧР – частичная регрессия, СТ – стабилизация, РО – рост опухоли.
Note: CR – complete regression, PR – partial regression, NC – no change, TG – tumor growth.

Conclusion

As EMPD is a relatively rare disease, there are no clear treatment recommendations in the literature based on large, multicenter studies comparing the efficacy of different therapies. PDT shows good results of the tumor treatment, both immediate (up to 40% of patients had complete regression of EMPD foci) and long-term (relapses in the case of 21% of patients at follow-up periods of 2-29 months; relapses in most cases occurred during the treatment of a large area foci). The advantages of PDT are the absence of systemic toxicity, non-invasiveness, selectivity of action, the absence of carcinogenic potential, the possibility of conducting several courses of treatment, and good cosmetic results. The highest efficiency of PDT was noted for small foci treatment (up to 4 cm^2).

REFERENCES

1. Ishizuki S., Nakamura Y. Extramammary Paget's Disease: Diagnosis, Pathogenesis, and Treatment with Focus on Recent Developments, *Curr Oncol,* 2021, vol. 28(4), pp. 2969-2986. doi: 10.3390/curroncol28040260.
2. Leong J.Y., Chung P.H. A primer on extramammary Paget's disease for the urologist, *Transl Androl Urol,* 2020, vol. 9(1), pp. 93-105. doi: 10.21037/tau.2019.07.14.
3. Shim P.J., Zeitouni N.C. Photodynamic therapy for extramam mary Paget's disease: A systematic review of the literature, *Photodiagnosis Photodyn Ther,* 2020, vol. 31, 101911. doi: 10.1016/j.pdpdt.2020.101911.
4. Jones R.E., Austin C., Ackerman A.B. Extramammary Paget's disease. A critical reexamination, *Am J Dermatopathol,* 1979, vol. 1(2), pp. 101-132. doi: 10.1097/00000372-197900120-00002.
5. Wagner G., Sachse M.M. Extramammary Paget disease – clinical appearance, pathogenesis, management, *Journal der Deutschen Dermatologischen Ges,* 2011, vol. 9(6), pp. 448-454. doi:10.1111/j.1610-0387.2010.07581.x.
6. Rioli D.I., Samimi M., Beneton N., Hainaut E., Martin L., Misery L., Quereux G. Efficacy and tolerance of photodynamic therapy for vulvar Paget's disease: a multicentric retrospective study, *Eur J Dermatol,* 2018, vol. 28(3), pp. 351-355. doi: 10.1684/ejd.2018.3289.
7. Kanitakis J. Mammary and extramammary Paget's disease, *J Euro Acad Dermatol Venereol*, 2007, vol. 21(5), pp. 581-90. doi: 10.1111/j.1468-3083.2007.02154.x.
8. Fardal R.W., Kierland R.R., Clagett O.T., Woolner L.B. Prognosis in cutaneous Paget's disease, *Postgrad Med,* 1964, vol. 36, pp. 584-93. doi: 10.1080/00325481.1964.11695362.
9. Siesling S., Elferink M.A.G., van Dijck J.A., Pierie J.P., Blokx W.A. Epidemiology and treatment of extramammary Paget disease in the Netherlands, *Eur J Surg Oncol,* 2007, vol. 33, pp. 951-955. doi: 10.1016/j.ejso.2006.11.028.
10. Chanda J.J. Extramammary Paget's disease: prognosis and relationship of internal malignancy, *J Am Acad Dermatol,* 1985, vol. 13, pp. 1009-14.
11. Herrel L.A., Weiss A.D., Goodman M., et al. Extramammary Paget's disease in males: survival outcomes in 495 patients, *Ann Surg Oncol,* 2015, vol. 22(5), pp. 1625-30. doi: 10.1245/s10434-014-4139-y.
12. Chung P.H., Kampp J.T., Voelzke B.B. Patients' Experiences with Extramammary Paget Disease: An Online Pilot Study Querying a Patient Support Group, *Urology,* 2018, vol. 111, pp. 214-219. doi: 10.1016/j.urology.2017.08.045.
13. Tanaka V.D., Sanches J.A., Torezan L., Niwa A.B., Festa Neto C. Mammary and extramammary Paget's disease: a study of 14 cases and the associated therapeutic difficulties, *Clinics (Sao Paulo, Brazil),* 2009, vol. 64(6), pp. 599-606. doi: 10.1590/s1807-59322009000600018.
14. Park S., Grossfeld G.D., McAninch J.W., et al. Extramammary Paget's disease of the penis and scrotum: excision, reconstruction and evaluation of occult malignancy, *J Urol,* 2001, vol. 166, pp. 2112-6; discussion 2117. doi: 10.1016/S0022-5347(05)65516-4.

ЛИТЕРАТУРА

1. Ishizuki S., Nakamura Y. Extramammary Paget's Disease: Diagnosis, Pathogenesis, and Treatment with Focus on Recent Developments // Curr Oncol. – 2021. – Vol. 28(4). – P. 2969-2986. doi: 10.3390/curroncol28040260.
2. Leong J.Y., Chung P.H. A primer on extramammary Paget's disease for the urologist // Transl Androl Urol. – 2020. – Vol. 9(1). – P. 93-105. doi: 10.21037/tau.2019.07.14.
3. Shim P.J., Zeitouni N.C. Photodynamic therapy for extramam mary Paget's disease: A systematic review of the literature // Photodiagnosis Photodyn Ther. – 2020. – Vol. 31. – 101911. doi: 10.1016/j.pdpdt.2020.101911.
4. Jones R.E., Austin C., Ackerman A.B. Extramammary Paget's disease. A critical reexamination // Am J Dermatopathol. – 1979. – Vol. 1(2). – P. 101-132. doi: 10.1097/00000372-197900120-00002.
5. Wagner G., Sachse M.M. Extramammary Paget disease – clinical appearance, pathogenesis, management // Journal der Deutschen Dermatologischen Ges. – 2011. – Vol. 9(6). – P. 448-454. doi:10.1111/j.1610-0387.2010.07581.x.
6. Rioli D.I., Samimi M., Beneton N., Hainaut E., Martin L., Misery L., Quereux G. Efficacy and tolerance of photodynamic therapy for vulvar Paget's disease: a multicentric retrospective study // Eur J Dermatol. – 2018. – Vol. 28(3). – P. 351-355. doi: 10.1684/ejd.2018.3289.
7. Kanitakis J. Mammary and extramammary Paget's disease // J Euro Acad Dermatol Venereol. –2007. – Vol. 21(5). – P. 581-90. doi: 10.1111/j.1468-3083.2007.02154.x.
8. Fardal R.W., Kierland R.R., Clagett O.T., Woolner L.B. Prognosis in cutaneous Paget's disease // Postgrad Med. –1964. – Vol. 36. – P. 584-93. doi: 10.1080/00325481.1964.11695362.
9. Siesling S., Elferink M.A.G., van Dijck J.A., Pierie J.P., Blokx W.A. Epidemiology and treatment of extramammary Paget disease in the Netherlands // Eur J Surg Oncol. – 2007. – Vol. 33. – P. 951-955. doi: 10.1016/j.ejso.2006.11.028.
10. Chanda J.J. Extramammary Paget's disease: prognosis and relationship of internal malignancy // J Am Acad Dermatol. –1985. – Vol. 13. – P. 1009-14.
11. Herrel L.A., Weiss A.D., Goodman M., et al. Extramammary Paget's disease in males: survival outcomes in 495 patients // Ann Surg Oncol. – 2015. – Vol. 22(5). – P. 1625-30. doi: 10.1245/s10434-014-4139-y.
12. Chung P.H., Kampp J.T., Voelzke B.B. Patients' Experiences with Extramammary Paget Disease: An Online Pilot Study Querying a Patient Support Group // Urology. – 2018. – Vol. 111. – P. 214-219. doi: 10.1016/j.urology.2017.08.045.
13. Tanaka V.D., Sanches J.A., Torezan L., Niwa A.B., Festa Neto C. Mammary and extramammary Paget's disease: a study of 14 cases and the associated therapeutic difficulties // Clinics (Sao Paulo, Brazil). – 2009. – Vol. 64(6). – P. 599-606. doi: 10.1590/s1807-59322009000600018.
14. Park S., Grossfeld G.D., McAninch J.W., et al. Extramammary Paget's disease of the penis and scrotum: excision, reconstruction and evaluation of occult malignancy // J Urol. – 2001. – Vol. 166. – P. 2112-6; discussion 2117. doi: 10.1016/S0022-5347(05)65516-4.

15. Nardelli A.A., Stafinski T., Menon D. Effectiveness of photodynamic therapy for mammary and extra-mammary Paget's disease: a state of the science review, *BMC Dermatol,* 2011, vol. 11, pp. 13. doi: 10.1186/1471-5945-11-13.
16. Morris C.R., Hurst E.A. Extramammary Paget's Disease: A Review of the Literature Part II: Treatment and Prognosis, *Dermatol Surg,* 2020, vol. 46(3), pp. 305-311. doi: 10.1097/DSS.0000000000002240.
17. Zollo J.D., Zeitouni N.C. The Roswell Park Cancer Institute experience with extramammary Paget's disease, *Br J Dermatol,* 2000, vol. 142(1), pp. 59-65.
18. Haberman H.F., Goodall J., Llewellyn M. Extramammary Paget's disease, *Can Med Assoc J,* 1978, vol. 118, pp. 161-162.
19. Kawatsu T., Miki Y. Triple extramammary Paget's disease, *Arch Dermatol,* 1971, vol. 104, pp. 316-319. doi: 10.1001/archderm.1971.04000210090018.
20. Watring W.G., Roberts J.A., Lagasse L.D., et al. Treatment of recurrent Paget's disease of the vulva with topical bleomycin, *Cancer,* 1978, vol. 41, pp. 10-11. doi: 10.1002/1097-0142(197801)41:1<10::AID-CNCR2820410103>3.0.CO;2-G.
21. Machida H., Moeini A., Roman L.D., et al. Effects of imiquimod on vulvar Paget's disease: a systematic review of literature, *Gynecol Oncol,* 2015, vol. 139, pp. 165-171. doi: 10.1016/j.ygyno.2015.07.097.
22. Louis-Sylvestre C., Haddad B., Paniel B.J. Paget's disease of the vulva: results of different conservative treatments, *Eur J Obstet Gynecol Reprod Biol,* 2001, vol. 99, pp. 253-255. doi: 10.1016/S0301-2115(01)00394-3.
23. Choi J.B., Yoon E.S., Yoon D.K., et al. Failure of carbon dioxide laser treatment in three patients with penoscrotal extramammary Paget's disease, *BJU Int,* 2001, vol. 88, pp. 297-298. doi: 10.1046/j.1464-410x.2001.02326.x.
24. Filonenko E.V., Ivanova-Radkevich V.I. Photodynamic therapy in the treatment of patients with mycosis fungoides, *Biomedical Photonics*, 2022, vol. 11, no. 1, pp. 27–36 (in Russian). doi: 10.24931/2413–9432–2022–11-1-27-36
25. Panaseykin Y.A., Filonenko E.V., Sevrukov F.E., Kapinus V.N., Polkin V.V., Isaev P.A., Kaprin A.D., Ivanov S.A. Possibilities of photodynamic therapy in the treatment of malignant tumors of the oral cavity, *Biomedical Photonics*, 2021, T. 10, No. 3, pp. 32–38 (in Russian). doi: 10.24931/2413–9432–2021–10–3–32–38
26. Gilyadova A.V., Romanko Yu.S., Ishchenko A.A., Samoilova S.V., Shiryaev A.A., Alekseeva P.M., Efendiev K.T., Reshetov I.V. Photodynamic therapy for precancer diseases and cervical cancer (review of literature), *Biomedical Photonics*, 2021, vol. 10, no. 4, pp. 59–67 (in Russian). doi: 10.24931/2413–9432–2021–10-4-59-67
27. Madan V., Loncaster J., Allan D., Lear J., Sheridan L., Leach C., et al. Extramammary Paget's disease treated with topical and systemic photodynamic therapy, *Photodiagnosis Photodyn. Ther,* 2005, vol. 2(4), pp. 309-311.
28. Shieh S., Dee A.S., Cheney R.T., Frawley N.P., Zeitouni N.C., Oseroff A.R. Photodynamic therapy for the treatment of extramammary Paget's disease, *Br. J. Dermatol,* 2002, vol. 146(6), pp. 1000-1005.
29. Wang X.L., Wang H.W., Guo M.X., Xu S.Z. Treatment of skin cancer and pre-cancer using topical ALA-PDT – a single hospital experience, *Photodiagnosis Photodyn. Ther,* 2008, vol. 5(2), pp. 127-133.
30. Fontanelli R., Papadia A., Martinelli F., Lorusso D., Grijuela B., Merola M., et al. Photodynamic therapy with M-ALA as non surgical treatment option in patients with primary extramammary Paget's disease, *Gynecol. Oncol,* 2013, vol. 130(1), pp. 90-94.
31. Li Q., Gao T., Jiao B., Qi X., Long H.A., Qiao H., et al. Long-term follow-up of in situ extramammary Paget's disease in Asian skin types IV/V treated with photodynamic therapy, *Acta Derm. Venereo Andrettal,* 2010, vol. 90(2), pp. 159-164.
32. Magnano M., Loi C., Bardazzi F., Burtica E.C., Patrizi A. Methyl – aminolevulinic acid photodynamic therapy and topical tretinoin

15. Nardelli A.A., Stafinski T., Menon D. Effectiveness of photodynamic therapy for mammary and extra-mammary Paget's disease: a state of the science review // BMC Dermatol. – 2011. – Vol. 11. – P. 13. doi: 10.1186/1471-5945-11-13.
16. Morris C.R., Hurst E.A. Extramammary Paget's Disease: A Review of the Literature Part II: Treatment and Prognosis // Dermatol Surg. – 2020. – Vol. 46(3). – P. 305-311. doi: 10.1097/DSS.0000000000002240.
17. Zollo J.D., Zeitouni N.C. The Roswell Park Cancer Institute experience with extramammary Paget's disease // Br J Dermatol. – 2000. – Vol. 142(1). – P. 59-65.
18. Haberman H.F., Goodall J., Llewellyn M. Extramammary Paget's disease // Can Med Assoc J. – 1978. – Vol. 118. – P. 161-162.
19. Kawatsu T., Miki Y. Triple extramammary Paget's disease // Arch Dermatol. – 1971. – Vol. 104. – P. 316-9. doi: 10.1001/archderm.1971.04000210090018.
20. Watring W.G., Roberts J.A., Lagasse L.D., et al. Treatment of recurrent Paget's disease of the vulva with topical bleomycin // Cancer. – 1978. – Vol. 41. – P. 10-1. doi: 10.1002/1097-0142(197801)41:1<10::AID-CNCR2820410103>3.0.CO;2-G.
21. Machida H., Moeini A., Roman L.D., et al. Effects of imiquimod on vulvar Paget's disease: a systematic review of literature // Gynecol Oncol. – 2015. – Vol. 139. – P. 165-71. doi: 10.1016/j.ygyno.2015.07.097.
22. Louis-Sylvestre C., Haddad B., Paniel B.J. Paget's disease of the vulva: results of different conservative treatments // Eur J Obstet Gynecol Reprod Biol. – 2001. – Vol. 99. – P. 253-5. doi: 10.1016/S0301-2115(01)00394-3.
23. Choi J.B., Yoon E.S., Yoon D.K., et al. Failure of carbon dioxide laser treatment in three patients with penoscrotal extramammary Paget's disease // BJU Int. – 2001. – Vol. 88. – P. 297-8. doi: 10.1046/j.1464-410x.2001.02326.x.
24. Филоненко Е.В., Иванова-Радкевич В.И. Фотодинамическая терапия в лечении больных грибовидным микозом // Biomedical Photonics. – 2022. – Т. 11, № 1. – С. 27–36. doi: 10.24931/2413–9432–2022–11-1-27-36
25. Панасейкин Ю.А., Филоненко Е.В., Севрюков Ф.Е., В.Н. Капинус, Полькин В.В., Исаев П.А., Каприн А.Д., Иванов С.А. Возможности фотодинамической терапии при лечении злокачественных опухолей полости рта // Biomedical Photonics. – 2021. – Т. 10, № 3. – С. 32–38. doi: 10.24931/2413–9432–2021–10–3–32–38
26. Гилядова А.В., Романко Ю.С., Ищенко А.А., Самойлова С.В., Ширяев А.А., Алексеева П.М., Эфендиев К.Т., Решетов И.В. Фотодинамическая терапия предраковых заболеваний и рака шейки матки (обзор литературы) // Biomedical Photonics. – 2021. – Т. 10, № 4. – С. 59–67. doi: 10.24931/2413–9432–2021–10-4-59-67
27. Madan V., Loncaster J., Allan D., Lear J., Sheridan L., Leach C., et al. Extramammary Paget's disease treated with topical and systemic photodynamic therapy // Photodiagnosis Photodyn. Ther. – 2005. – Vol. 2(4). – P. 309-311.
28. Shieh S., Dee A.S., Cheney R.T., Frawley N.P., Zeitouni N.C., Oseroff A.R. Photodynamic therapy for the treatment of extramammary Paget's disease // Br. J. Dermatol. – 2002. – Vol. 146(6). – P. 1000-1005.
29. Wang X.L., Wang H.W., Guo M.X., Xu S.Z. Treatment of skin cancer and pre-cancer using topical ALA-PDT – a single hospital experience // Photodiagnosis Photodyn. Ther. – 2008. – Vol. 5(2). – P. 127-133.
30. Fontanelli R., Papadia A., Martinelli F., Lorusso D., Grijuela B., Merola M., et al. Photodynamic therapy with M-ALA as non surgical treatment option in patients with primary extramammary Paget's disease // Gynecol. Oncol. – 2013. – Vol. 130(1). – P. 90-94.
31. Li Q., Gao T., Jiao B., Qi X., Long H.A., Qiao H., et al. Long-term follow-up of in situ extramammary Paget's disease in Asian skin types IV/V treated with photodynamic therapy // Acta Derm. Venereo Andrettal. – 2010. – Vol. 90(2). – P. 159-164.
32. Magnano M., Loi C., Bardazzi F., Burtica E.C., Patrizi A. Methyl – aminolevulinic acid photodynamic therapy and topical tretinoin in

in a patient with vulvar extramammary Paget's disease, *Dermatol. Ther,* 2013, vol. 26(2), pp. 170-172.

33. Raspagliesi F., Fontanelli R., Rossi G., Ditto A., Solima E., Hanozet F., et al. Photodynamic therapy using a methyl ester of 5-aminolevulinic acid in recurrent Paget's disease of the vulva: a pilot study, *Gynecol. Oncol,* 2006, vol. 103(2), pp. 581-586.
34. Runfola M.A., Weber T.K., Rodriguez-Bigas M.A., Dougherty T.J., Petrelli N.J. Photodynamic therapy for residual neoplasms of the perianal skin, *Dis. Colon Rectum,* 2000, vol. 43(4), pp. 499-502.
35. Housel J.P., Izikson L., Zeitouni N.C. Noninvasive extramammary Paget's disease treated with photodynamic therapy: case series from the Roswell Park Cancer Institute, *Dermatol. Surg,* 2010, vol. 36(11), pp. 1718-1724.
36. Kitagawa K.H., Bogner P., Zeitouni N.C. Photodynamic therapy with methyl-aminolevulinate for the treatment of double extramammary Paget's disease, *Dermatol. Surg,* 2011, vol. 37(7), pp. 1043-1046.
37. Al Yousef A., Boccara O., Moyal-Barracco M., Zimmermann U., Saiag P. Incomplete efficacy of 5-aminolevulinic acid (5 ALA) photodynamic therapy in the treatment of widespread extramammary Paget's disease, *Photodermatol. Photoimmunol. Photomed,* 2012, vol. 28(1), pp. 53-55.
38. Calzavara-Pinton P.G., Rossi M.T., Sala R. A retrospective analysis of real-life practice of off-label photodynamic therapy using methyl aminolevulinate (MALPDT) in 20 Italian dermatology departments. Part 2: oncologic and infectious indications, *Photochem. Photobiol. Sci,* 2013, vol. 12(1), pp. 158-165.
39. Wang D., Wang P., Li C., Zhou Z., Zhang L., Zhang G., Wang X. Efficacy and safety of HpD-PDT for Extramammary Paget's Disease refractory to conventional therapy: A prospective, open-label and single arm pilot study, *Photodiagnosis Photodyn Ther,* 2022, vol. 37, 102670. doi: 10.1016/j.pdpdt.2021.102670.

a patient with vulvar extramammary Paget's disease // Dermatol. Ther. – 2013. – Vol. 26(2). – P. 170-172.

33. Raspagliesi F., Fontanelli R., Rossi G., Ditto A., Solima E., Hanozet F., et al. Photodynamic therapy using a methyl ester of 5-aminolevulinic acid in recurrent Paget's disease of the vulva: a pilot study // Gynecol. Oncol. – 2006. – Vol. 103(2). – P. 581-586.
34. Runfola M.A., Weber T.K., Rodriguez-Bigas M.A., Dougherty T.J., Petrelli N.J. Photodynamic therapy for residual neoplasms of the perianal skin // Dis. Colon Rectum. – 2000. – Vol. 43(4). – P. 499-502.
35. Housel J.P., Izikson L., Zeitouni N.C. Noninvasive extramammary Paget's disease treated with photodynamic therapy: case series from the Roswell Park Cancer Institute // Dermatol. Surg. – 2010. – Vol. 36(11). – P. 1718-1724.
36. Kitagawa K.H., Bogner P., Zeitouni N.C. Photodynamic therapy with methyl-aminolevulinate for the treatment of double extramammary Paget's disease // Dermatol. Surg. – 2011. – Vol. 37(7). – P. 1043-1046.
37. Al Yousef A., Boccara O., Moyal-Barracco M., Zimmermann U., Saiag P. Incomplete efficacy of 5-aminolevulinic acid (5 ALA) photodynamic therapy in the treatment of widespread extramammary Paget's disease // Photodermatol. Photoimmunol. Photomed. – 2012. – Vol. 28(1). – P. 53-55.
38. Calzavara-Pinton P.G., Rossi M.T., Sala R. A retrospective analysis of real-life practice of off-label photodynamic therapy using methyl aminolevulinate (MALPDT) in 20 Italian dermatology departments. Part 2: oncologic and infectious indications // Photochem. Photobiol. Sci. – 2013. – Vol. 12(1). – P. 158-165.
39. Wang D., Wang P., Li C., Zhou Z., Zhang L., Zhang G., Wang X. Efficacy and safety of HpD-PDT for Extramammary Paget's Disease refractory to conventional therapy: A prospective, open-label and single arm pilot study // Photodiagnosis Photodyn Ther. – 2022. – Vol. 37. – 102670. doi: 10.1016/j.pdpdt.2021.102670.

MODERN ASPECTS OF PHOTODYNAMIC THERAPY OF BASAL CELL SKIN CANCER

Reshetov I.V.[1,2,3], **Korenev S.V.**[4], **Romanko Yu.S.**[1,2]
[1]Sechenov First Moscow State Medical University, Moscow, Russia
[2]FSBF FRCC of the FMBA, Moscow, Russia
[3]Moscow Witte University, Moscow, Russia
[4]Immanuel Kant Baltic Federal University, Kaliningrad, Russia

Abstract

Photodynamic therapy (PDT) is one of the most effective treatments for basal cell skin cancer (BCC). As the incidence rate of BCC is increasing worldwide, interest in developing new methods for diagnosing and treating this disease, taking into account long-term cosmetic results, is growing. The review article presents the results of domestic and foreign studies on the treatment of BCC with PDT. The presented results of studies from various domestic and foreign clinics indicate the high efficiency of independent PDT and a combination of PDT with other treatment methods. PDT is proposed to be used in combination with surgical methods and radiation therapy, immunomodulating and chemotherapeutic agents, and inhibitors of molecules involved in the carcinogenic process. These new strategies open the way to increasing the effectiveness of treatment and prevention of BCC. Moreover, in all studies, the safety of this non-invasive treatment, a low level of adverse reactions during therapy, good tolerance for the patient and excellent cosmetic treatment results are noted. The guidelines currently being developed in Europe and the United States provide consistent expert advice that reflects current published evidence of treatment outcomes for BCC using PDT. Moreover, the recommendations emphasize that the treatment plan for patients with "difficult to treat" BCC should be discussed at an interdisciplinary oncological council.

Key words: basal cell cancer, photodynamic therapy, photosensitizer, photogem, photosens, photoditazine, photolon, aminolevulinic acid.

For citations: Reshetov I.V., Korenev S.V., Romanko Yu.S. Modern aspects of photodynamic therapy of basal cell skin cancer, *Biomedical Photonics*, 2022, vol. 11, no. 3, pp. 35–39. doi: 10.24931/2413–9432–2022–11-3-35–39.

Contacts: Korenev S.V., e-mail: korenevsv@mail.ru

СОВРЕМЕННЫЕ АСПЕКТЫ ФОТОДИНАМИЧЕСКОЙ ТЕРАПИИ ПРИ БАЗАЛЬНОКЛЕТОЧНОМ РАКЕ КОЖИ

И.В. Решетов[1,2,3], **С.В. Коренев**[4], **Ю.С. Романко**[1,2]
[1]ФГАОУ ВО Первый МГМУ им. И.М. Сеченова Минздрава России, Москва, Россия
[2]Академия постдипломного образования ФГБУ ФНКЦ ФМБА, Москва, России
[3]ЧОУВО «МУ им. С.Ю. Витте», Москва, Россия
[4]ФГАОУ ВО «БФУ им. И. Канта», Калининград, Россия

Резюме

Фотодинамическая терапия (ФДТ) является одним из наиболее эффективных методов лечения базальноклеточного рака кожи (БКРК). По мере роста показателей заболеваемости БКРК во всём мире всё больше возрастает интерес к разработке новых методов диагностики и лечения данного заболевания с учётом отдалённых косметических результатов. В обзорной статье приводятся результаты отечественных и зарубежных исследований по лечению БКРК с помощью ФДТ. Представленные результаты исследований различных отечественных и зарубежных клиник свидетельствуют о высокой эффективности самостоятельной ФДТ и комбинации ФДТ с другими методами лечения. ФДТ предлагается применять в сочетании с хирургическими методами и лучевой терапией, иммуномодулирующими и химиотерапевтическими агентами, ингибиторами молекул, участвующих в канцерогенном процессе. Эти новые стратегии открывают путь к повышению эффективности лечения и профилактики БКРК. При этом во всех исследованиях отмечается безопасность данного неинвазивного лечения, низкий уровень побочных реакций при проведении терапии, хорошая переносимость для пациента и превосходные косметические результаты лечения. В разработанных в настоящее время в Европе и США руководствах представлены согласованные экспертные рекомендации, отражающие текущие опубликованные доказательства результативности лечения БКРК с использованием ФДТ. При этом в рекомендациях подчеркивается, что план терапии пациентов с трудно поддающимися лечению БКРК должен обсуждаться на междисциплинарном онкологическом совете.

Ключевые слова: базальноклеточный рак кожи, фотодинамическая терапия, фотосенсибилизатор, фотогем, фотосенс, фотодитазин, фотолон, аминолевулиновая кислота.

Для цитирования: Решетов И.В., Коренев С.В., Романко Ю.С. Современные аспекты фотодинамической терапии при базальноклеточном раке кожи // Biomedical Photonics. – 2022. – Т. 11, № 3. – С. 35–39. doi: 10.24931/2413–9432–2022–11-3-35–39.

Контакты: Коренев С.В., e-mail: korenevsv@mail.ru

Introduction

The incidence of skin cancer is growing widespread in the world [1] in general, as well as the incidence of the most common human cancer such as basal cell skin cancer (BCC) continues to increase [2]. The problem of treating BCC is also relevant for the Russian Federation, which is primarily due to a very high level of incidence. This disease is characterized by a recurrent nature of the course, frequent localization in open areas of the skin with a predominance of facial skin lesions, insufficient effectiveness of existing treatment methods, and significant cosmetic defects. The main methods of treatment of BCC are surgical method, radiation therapy, cryodestruction, immunotherapy, diathermocoagulation [3-12].

Anyway, the above-mentioned methods of treatment have a number of disadvantages: significant side effects, limitations in the case of repeated treatment, not always sufficiently effective and organ-preserving treatment results. Currently, photodynamic therapy (PDT) is one of the most effective, minimally invasive, and organ-preserving methods of treating BCC [3].

A strong inflammatory reaction and PDT-activated immune defense of the body against tumors represent one of the central events in the mechanism of tumor destruction. They include a complex series of interacting specific and nonspecific reactions of different cell types capable of effectively destroying tumor cells by cytolytic and apoptotic mechanisms [13].

In recent years, the PDT method has been actively developed [14]. According to many colleagues, PDT is an effective and safe non–invasive treatment of BCC with excellent cosmetic results. The effectiveness of PDT in BCC is confirmed by extensive studies and clinical trials [15, 16].

PDT in BCC treatment

A group of Russian scientists presented the results of PDT of 139 patients with BCC in their work in 2016. A study of the effectiveness and safety of PDT was conducted using four photosensitizers (FS): photohem, photosense, photolone and photoditazine. It was shown that PDT with the use of chlorin series FS (photolone and photoditazine) provided better long-term results, improving the relapse-free 3-year survival rate up to 90.4% and 92.3%, respectively, compared with 54.7% and 71.1% in groups in which treatment was carried out using photohem and photosense. At the same time, the authors did not indicate any complications after PDT [17].

In 2019, the PDT subgroup of the steering committee of the European Dermatology Forum prepared a guide that reviewed all current approved protocols and emerging new indications for the PDT use in the treatment of actinic keratosis, Bowen's disease, and BCC. The manual presents agreed expert recommendations reflecting the current published evidence of the PDT effectiveness in the treatment of BCC [18].

Multidisciplinary experts from European Dermatological Forum, European Association of Dermato-Oncology and European Organization of Research and Treatment of Cancer jointly developed recommendations for the diagnosis and treatment of BCC. In accordance with these recommendations, PDT is an effective treatment for superficial and nodular forms of BCC. At the same time, other alternative methods are recommended for the treatment of other forms of BCC. According to the recommendations, the treatment plan for patients with "difficult to treat" BCC should be discussed at the interdisciplinary oncological council [19].

The high efficiency of PDT in the treatment of superficial and nodular forms is indicated by numerous authors [20-23].

K.C. Blanco et al. also showed high efficiency of treatment of superficial and nodular forms of BCC with the use of PDT during a multicenter clinical trial conducted between 2012 and 2014. They also provided the analysis of adverse reactions during and after PDT according to a standardized protocol of the treatment of 866 cases of BCC. In total, 728 patients with a confirmed clinical and histopathological diagnosis of BCC underwent treatment. The size of the tumors was up to 2 cm in diameter. Treatment included curettage and topical application of a cream containing 20% methyl ester of 5-aminolevulinate followed by PDT (radiation wavelength was 630 nm, dose rate was 150 J/cm^2), which was carried out 3 hours after applying the cream. The intensity of pain sensations during and after treatment was assessed. The intensity correlated with the anatomical localization of the lesion. Patients reported a higher intensity of pain in the lesion focus located on the head and the neck. The pain was less intense on the trunk and the limbs. The

number of sessions also affected the pain reaction. The authors emphasize that PDT occupies a significant place in the treatment of BCC due to its low cost, ease of use and low level of side effects [24].

A number of researchers recommend using PDT in the treatment of BCC if surgical treatment cannot be carried out for some reasons [25-27].

PDT has the advantage of being a non-invasive treatment, providing high efficiency and optimal cosmetic results even though, currently, surgical treatment is most often used in the treatment of this pathology in the world. However, resistant or recurrent tumors may appear, becoming sometimes more aggressive. Therefore, increasing the effectiveness of PDT by combining it with other therapeutic methods is an interesting area of research. Depending on the characteristics and type of tumor, PDT can be used combined with immunomodulatory (imiquimod) and chemotherapeutic (5-fluorouracil, methotrexate, diclofenac, etc.) agents, inhibitors of certain molecules involved in the carcinogenic process, surgical methods, and radiation therapy. These new strategies pave the way to improving the treatment effectiveness and BCC prevention [28].

PDT is an alternative to surgical therapy in the treatment of BCC. Nevertheless, PDT is mainly used in the treatment of superficial tumors nowadays. According to researchers from the University of Bologna, combined treatment in the form of pretreatment of BCC with ablative lasers can increase the effectiveness of therapy. They evaluated a combination therapy using PDT and a CO_2 laser for the treatment of superficial and nodular BCC. In the presented prospective monocentric study, patients with BCC received therapy using a continuous superimpulsive CO_2 laser for the treatment of the nodular form of BCC and a fractional CO_2 laser in the case of the surface form of BCC. Subsequently, all patients underwent PDT using a 5-aminolevulinic acid methyl ester cream and an Aktilite CL128® lamp (Galderma). 32 patients (20 men, 12 women) aged 45 to 96 years (a total of 181 foci of BCR) were cured with a CO_2 laser combined with PDT. After 3 months, 100% cure was achieved, there were no signs of relapse at follow-up from 4 to 18 months in 97.2% of cases. Mild adverse reactions and good aesthetic results were observed. The authors recommend this combination therapy due to its high efficiency, good aesthetic results and a small number of side effects [29].

X. Li et al. also propose the development of combined treatment using PDT. In their study, 8 patients with periorbital BCC received PDT using 5-aminolevulinic acid combined with surgery. After tumor removal (mainly with the preservation of normal skin tissue), each area of the tumor was irradiated using laser radiation with a wavelength of 635 nm and a power density of 177 J/cm^2 for 15 minutes. In total, 3 PDT courses were conducted during and after surgery. BCC was confirmed by histological examination. The structure of the tumor tissues was studied using transmission electron microscopy, which showed that PDT has an inhibitory effect on the growth of BCC, causing necrosis of tumor cells. Subsequently, there were no relapses during dynamic follow-up for up to 5 years. Patients with infiltrative orbital BCC were able to complete the treatment protocol with good cosmetic results and without significant side effects. The authors note that PDT combined with limited surgical intervention is a safe, effective, and minimally invasive method of treating orbital BCC. At the same time, PDT during and after surgery can help to reduce the volume of surgical intervention [30].

Conclusion

The scope of PDT application continues to expand. Currently, there are quite a lot of data that demonstrate the effectiveness of PDT in the treatment of various forms of BCC. PDT shows high efficiency, good tolerability of therapy by patients and excellent cosmetic results. Considering that pain remains the most common side effect, various effective strategies aimed at reducing pain are currently being developed [31].

Many prospective studies have shown an increase in the effectiveness of the treatment of BCC with the combined use of PDT and other treatment methods.

REFERENCES

1. Baykalova O.I., Belyaev A.M., Prokhorov G.G., Radzhabova Z.A. Treatment of squamous cell carcinoma of the skin with the use og cryogenic technologies, *Siberian journal of oncology*, 2020, vol. 19, no. 6, pp. 99–105. (In Russian).
2. Cameron M.C., Lee E., Hibler B.P., Barker C., Mori S., Cordova M., Nehal K.S., Rossi A.M. Basal cell carcinoma: Epidemiology; pathophysiology; clinical and histological subtypes; and disease associations, *J Am Acad Dermatol*, 2019, vol. 80(2), pp. 303-317.
3. Filonenko E.V., Serova L.G. Photodynamic therapy in clinical practice, *Biomedical Photonics*, 2016, vol. 5, no. 2, pp. 26–37 (in Russian).
4. Belova I.A. Risk of local relapse, metastases, new primary tumors, and cosmetic defects in patients with malignant skin tumors.

ЛИТЕРАТУРА

1. Байкалова О.И., Беляев А.М., Прохоров Г.Г., Раджабова З.А. Лечение плоскоклеточного рака кожи с применением криогенных технологий // Сибирский онкологический журнал. – 2020. – Т.19, № 6. – С. 99–105.
2. Cameron M.C., Lee E., Hibler B.P., Barker C., Mori S., Cordova M., Nehal K.S., Rossi A.M. Basal cell carcinoma: Epidemiology; pathophysiology; clinical and histological subtypes; and disease associations // J Am Acad Dermatol. – 2019. – Vol. 80(2). – P. 303-317.
3. Филоненко Е.В., Серова Л.Г. Фотодинамическая терапия в клинической практике // Biomedical Photonics. – 2016. – Т. 5, № 2. – С. 26–37.
4. Белова И.А. Риски образования рецидивов, метастазов, новых первичных видов рака и косметических дефектов при

German experience of their prevention, *Head and neck. Russian Journal*, 2013, no. 1, pp. 25–39 (in Russian).
5. Belova I.A. Methods of microscopically controlled surgery (review), *Head and neck. Russian Journal*, 2013, no. 3, pp. 22–34 (in Russian).
6. Polyakov P.Y., Oltarzhevskaya N.D., Bychenkov O.A., Korovina M.A. Radiotherapy of skin carcinoma with directed application of metronidazol as a radiosensitizing agent, *Head and neck. Russian Journal*, 2013, no. 1, pp. 14–18 (in Russian).
7. Breuninger H., Belova I. Instruction for conducting microscopically controlled surgery and threedimensional hystology for removal of malignant skin lesions, *Head and neck. Russian Journal*, 2017, no. 4, pp. 62–72 (in Russian).
8. Pustynskiy I.N., Paches A.I., Tkachev S.I., Kropotov M.A., Alieva S.B., Yagubov A.S., Bazhutova G.A., Slanina S.V. Cryoradiotherapy for patients with locally advanced buccal skin cancer, *Siberian Journal of Oncology*, 2013, no. 6, pp. 5–8 (in Russian).
9. Prokhorov G.G., Galunova T.Yu., Radzhabova Z.A., Madagov A.S., Kotov M.A., Nazhmudinov R.A., Rakitina D.A., Artemiev S.S. Microflora of infiltrative-ulcerative form of basal cell skin cancer against the background of cryogenic treatment, *Vopr Onkol*, 2017, vol. 63, no. 3, pp. 486-489 (in Russian).
10. Molochkov A.V., Rumyantsev S.A., Khlebnikova A.N. Enhancement of interferon treatment for large basaliomas, *Almanac of Clinical Medicine*, 2017, vol. 45, no. 4, pp. 314-320 (in Russian).
11. Pustynskiy I.N., Tabolinovskaya T.D., Tkachev S.I., Alieva S.B., Azizian R.I., Kiva E.V., Egorova A.V., Peterson S.B. The treatment of patients with locally-advanced recurrent skin cancer of the face by cryo-radiotherapy, *Siberian Journal of Oncology*, 2017, vol. 16, no. 6, pp. 67–72 (in Russian).
12. Shaikhaliev A.I., Petruk P.S., Arazashvili L.D., Polyakov K.A., Cherkesov I.V., Kolobovnikova A.I. New approaches in basal cell carcinoma treatment. Case report, *Head and neck. Russian Journal*, 2018, no. 2, pp. 45–49 (in Russian).
13. Yuzhakov V.V., Burmistrova N.V., Fomina N.K., Bandurko L.N., Sevanjkaeva L.E., Starovoytova A.V., Yakovleva N.D., Tsyganova M.G., Ingelj I.E., Ostroverkhov P.V., Kaplan M.A., Grin M.A., Mazhuga A.G., Mironov A.F., Galkin V.N., Romanko Yu.S. Morphofunctional characteristics of rat sarcoma M-1 after photodynamic therapy with the bacteriochlorophyll a derivative, *Biomedical Photonics*, 2016, vol. 5, no. 4, pp. 4-14 (in Russian).
14. Filonenko E.V. Clinical implementation and scientific development of photodynamic therapy in Russia in 2010-2020. *Biomedical Photonics*, 2021, Vol. 10(4), pp. 4-22. doi: 10.24931/2413-9432-2021-9-4-4-22
15. Reshetov I.V., Korenev S.V., Romanko Yu.S. Forms of cell death and targets at photodynamic therapy, *Siberian Journal of Oncology*, 2022, vol. 21, no. 5, pp. 149–154.
16. Fargnoli M.C., Peris K. Photodynamic therapy for basal cell carcinoma, *Future Oncol,* 2015, vol. 11(22), pp. 2991-2996.
17. Romanko Y.S., Kaplan M.A., Ivanov S.A., Galkin V.N., Molochkova Y.V., Kuntsevich Z.S., Tretiakova E.I., Sukhova T.E., Molochkov V.A., Molochkov A.V. Efficacy of photodynamic therapy for basal cell carcinoma using photosensitizers of different classes, *Vopr Onkol*, 2016, vol. 62, no. 3, pp. 447-50 (in Russian).
18. Morton C.A., Szeimies R.M., Basset-Seguin N., Calzavara-Pinton P., Gilaberte Y., Haedersdal M., Hofbauer G.F.L., Hunger R.E., Karrer S., Piaserico S., Ulrich C., Wennberg A.M., Braathen L.R. European Dermatology Forum guidelines on topical photodynamic therapy 2019 Part 1: treatment delivery and established indications – actinic keratoses, Bowen's disease and basal cell carcinomas, *J Eur Acad Dermatol Venereol,* 2019, vol. 33(12), pp. 2225-2238.
19. Peris K., Fargnoli M.C., Garbe C., Kaufmann R., Bastholt L., Seguin N.B., Bataille V., Marmol V.D., Dummer R., Harwood

злокачественных новообразованиях кожи и немецкий опыт их предотвращения // Голова и шея = Head and neck. Russian Journal. – 2013. –№ 1. – С. 25–39.
5. Белова И.А. Методы микроскопически контролируемой хирургии (обзор литературы) // Голова и шея = Head and neck. Russian Journal. – 2013. –№ 3. – С. 22–34.
6. Поляков П.Ю., Олтаржевская Н.Д., Быченков О.А., Коровина М.А. Лучевая терапия рака кожи с направленным подведением радиосенсибилизатора метронидазола // Голова и шея = Head and neck. Russian Journal. – 2013. –№ 1. – С. 14–18.
7. Бройнингер Х., Белова И. Инструкция к проведению микроскопически контролируемой хирургии и трехмерной гистологии для удаления злокачественных новообразований кожи // Голова и шея = Head and neck. Russian Journal. – 2017. –№ 4. – С. 62–72.
8. Пустынский И.Н., Пачес А.И., Ткачев С.И., Кропотов М.А., Алиева С.Б., Ягубов А.С., Бажутова Г.А., Сланина С.В. Криолучевое лечение больных местнораспространенным раком кожи щеки // Сибирский онкологический журнал. – 2013. – № 6. – С. 5-8.
9. Прохоров Г.Г., Галунова Т.Ю., Раджабова З.А., Мадагов А.С., Котов М.А., Нажмудинов Р.А., Ракитина Д.А., Артемьев С.С. Микрофлора инфильтративно-язвенной формы базальноклеточного рака кожи на фоне криогенного лечения // Вопросы онкологии. – 2017. – Т. 63, № 3. – С. 486–489.
10. Молочков А.В., Румянцев С.А., Хлебникова А.Н. Совершенствование интерферонотерапии базалиом больших размеров // Альманах клинической медицины. – 2017. – Т. 45, № 4. – С. 314–320.
11. Пустынский И.Н., Таболиновская Т.Д., Ткачев С.И., Алиева С.Б., Азизян Р.И., Кива Е.В., Егорова А.В., Петерсон С.Б. Лечение больных с местнораспространенными рецидивами рака кожи лица крио-лучевым методом // Сибирский онкологический журнал. – 2017. – Т. 16, № 6. – С. 67–72.
12. Шайхалиев А.И., Петрук П.С., Аразашвили Л.Д., Поляков К.А., Черкесов И.В., Колобовникова А.И. Новые подходы в лечении базалиомы. Клинический случай // Голова и шея = Head and neck. Russian Journal. – 2018. –№ 2. – С. 45–49.
13. Южаков В.В., Бурмистрова Н.В., Фомина Н.К., Бандурко Л.Н., Севанькаева Л.Е., Старовойтова А.В., Яковлева Н.Д., Цыганова М.Г., Ингель И.Э., Островерхов П.В., Каплан М.А., Грин М.А., Мажуга А.Г., Миронов А.Ф., Галкин В.Н., Романко Ю.С. Морфофункциональные характеристики саркомы М-1 крыс после фотодинамической терапии с производным бактериохлорофилла а // Biomedical Photonics. – 2016. – Т. 5, № 4. – С. 4-14.
14. Филоненко Е.В. Клиническое внедрение и научное развитие фотодинамической терапии в России в 2010-2020 гг. // Biomedical Photonics. – 2021. – Т. 10, № 4. – С. 4–22. doi: 10.24931/2413-9432-2021-9-4-4-22
15. Решетов И.В., Коренев С.В., Романко Ю.С. Формы гибели клеток и мишени при фотодинамической терапии // Сибирский онкологический журнал. – 2022. – Т. 21, № 5. – С. 149–154.
16. Fargnoli M.C., Peris K. Photodynamic therapy for basal cell carcinoma // Future Oncol. – 2015. – Vol. 11(22). – P. 2991-2996.
17. Романко Ю.С., Каплан М.А., Иванов С.А., Галкин В.Н., Молочкова Ю.В., Кунцевич Ж.С., Третьякова Е.И., Сухова Т.Е., Молочков В.А., Молочков А.В. Эффективность фотодинамической терапии базально-клеточной карциномы с использованием фотосенсибилизаторов различных классов // Вопросы онкологии. – 2016. – Т. 62, № 3. – С. 447-450.
18. Morton C.A., Szeimies R.M., Basset-Seguin N., Calzavara-Pinton P., Gilaberte Y., Haedersdal M., Hofbauer G.F.L., Hunger R.E., Karrer S., Piaserico S., Ulrich C., Wennberg A.M., Braathen L.R. European Dermatology Forum guidelines on topical photodynamic therapy 2019 Part 1: treatment delivery and established indications – actinic keratoses, Bowen's disease and basal cell carcinomas // J Eur Acad Dermatol Venereol. – 2019. – Vol. 33(12). – P. 2225-2238.
19. Peris K., Fargnoli M.C., Garbe C., Kaufmann R., Bastholt L., Seguin N.B., Bataille V., Marmol V.D., Dummer R., Harwood C.A., Haus-

C.A., Hauschild A., Höller C., Haedersdal M., Malvehy J., Middleton M.R., Morton C.A., Nagore E., Stratigos A.J., Szeimies R.M., Tagliaferri L., Trakatelli M., Zalaudek I., Eggermont A., Grob J.J.; European Dermatology Forum (EDF), the European Association of Dermato-Oncology (EADO) and the European Organization for Research and Treatment of Cancer (EORTC). Diagnosis and treatment of basal cell carcinoma: European consensus-based interdisciplinary guidelines, *Eur J Cancer,* 2019, vol. 118, pp. 10-34.

20. Morton C., Szeimies R.M., Sidoroff A., Wennberg A.M., Basset-Seguin N., Calzavara-Pinton P., Gilaberte Y., Hofbauer G., Hunger R., Karrer S., Lehmann P., Piaserico S., Ulrich C., Braathen L.; European Dermatology Forum. European Dermatology Forum Guidelines on topical photodynamic therapy, *Eur J Dermatol,* 2015, vol. 25(4), pp. 296-311.
21. Ozog D.M., Rkein A.M., Fabi S.G., Gold M.H., Goldman M.P., Lowe N.J., Martin G.M., Munavalli G.S. Photodynamic Therapy: A Clinical Consensus Guide therapy, *Dermatol Surg,* 2016., vol. 42(7), pp. 804-827.
22. Savoia P., Deboli T., Previgliano A., Broganelli P. Usefulness of Photodynamic Therapy as a Possible Therapeutic Alternative in the Treatment of Basal Cell Carcinoma, *Int J Mol Sci,* 2015, vol. 16(10), pp. 23300-23317.
23. Morton C.A. A synthesis of the world's guidelines on photodynamic therapy for non-melanoma skin cancer, *Eur J Cancer,* 2018, vol. 153(6), pp. 783-792.
24. Blanco K.C., Inada N.M., Silva A.P., Stringasci M.D., Buzzá H.H., Ramirez D.P., Sálvio A.G., Moriyama L.T., Kurachi C., Bagnato V.S. A Multicenter Clinical Study of Expected and Unexpected Side Reactions During and After Skin Cancer Treatment by Photodynamic Therapy, *Skinmed,* 2017, vol. 15(2), pp. 113-118.
25. Filonenko E.V., Grigoryevykh N.I., Ivanova-Radkevich V.I. Photodynamic therapy of a patient with basal cell skin cancer of the ear stage T3N0M0 (clinical case), *Biomedical Photonics*, 2021, vol. 10, no. 4, pp. 68–70 (in Russian).
26. Paoli J., Gyllencreutz J.D., Fougelberg J., Backman E.J., Modin M., Polesie S., Zaar O. Nonsurgical Options for the Treatment of Basal Cell Carcinoma, *Dermatol Pract Concept,* 2019, vol. 9(2), pp. 75-81.
27. Kaplan M.A., Romanko Y.S. Photodynamic therapy as a new radical treatment for patients with recurrent tumors, *Vopr Onkol*, 2000, vol. 46, no. 2, p. 238 (in Russian).
28. Lucena S.R., Salazar N., Gracia-Cazaña T., Zamarrón A., González S., Juarranz Á., Gilaberte Y. Combined Treatments with Photodynamic Therapy for Non-Melanoma Skin Cancer, *Int J Mol Sci*, 2015, vol. 16(10), pp. 25912-25933.
29. Ferrara F., Lacava R., Barisani A., Messori S., Patrizi A., Bardazzi F., Vaccari S. Combined CO2 laser and photodynamic therapy enhances the efficacy of treatment of basal cell carcinomas, *J Dtsch Dermatol Ges*, 2019, vol. 17(12), pp. 1251-1256.
30. Li X., Tan L., Kou H., Zhang J., Wang Y., Li G., Lu Y. Ocular preservation through limited tumor excision combined with ALA-PDT in patients with periocular basal cell carcinoma, *Photodiagnosis Photodyn Ther*, 2019, vol. 27, pp. 291-294.
31. Zeitouni N.C., Sunar U., Rohrbach D.J., Paquette A.D., Bellnier D.A., Shi Y., Wilding G., Foster T.H., Henderson B.W. A prospective study of pain control by a 2-step irradiance schedule during topical photodynamic therapy of nonmelanoma skin cancer, Dermatol Surg., 2014, vol. 40(12), pp. 1390-1394.

child A., Höller C., Haedersdal M., Malvehy J., Middleton M.R., Morton C.A., Nagore E., Stratigos A.J., Szeimies R.M., Tagliaferri L., Trakatelli M., Zalaudek I., Eggermont A., Grob J.J.; European Dermatology Forum (EDF), the European Association of Dermato-Oncology (EADO) and the European Organization for Research and Treatment of Cancer (EORTC). Diagnosis and treatment of basal cell carcinoma: European consensus-based interdisciplinary guidelines // Eur J Cancer. – 2019. – Vol. 118. – P. 10-34.

20. Morton C., Szeimies R.M., Sidoroff A., Wennberg A.M., Basset-Seguin N., Calzavara-Pinton P., Gilaberte Y., Hofbauer G., Hunger R., Karrer S., Lehmann P., Piaserico S., Ulrich C., Braathen L.; European Dermatology Forum. European Dermatology Forum Guidelines on topical photodynamic therapy // Eur J Dermatol. – 2015. – Vol. 25(4). – P. 296-311.
21. Ozog D.M., Rkein A.M., Fabi S.G., Gold M.H., Goldman M.P., Lowe N.J., Martin G.M., Munavalli G.S. Photodynamic Therapy: A Clinical Consensus Guide therapy // Dermatol Surg. – 2016. – Vol. 42(7). – P. 804-827.
22. Savoia P., Deboli T., Previgliano A., Broganelli P. Usefulness of Photodynamic Therapy as a Possible Therapeutic Alternative in the Treatment of Basal Cell Carcinoma // Int J Mol Sci. – 2015. – Vol. 16(10). – P. 23300-23317.
23. Morton C.A. A synthesis of the world's guidelines on photodynamic therapy for non-melanoma skin cancer // Eur J Cancer. – 2018. – Vol. 153(6). – P. 783-792.
24. Blanco K.C., Inada N.M., Silva A.P., Stringasci M.D., Buzzá H.H., Ramirez D.P., Sálvio A.G., Moriyama L.T., Kurachi C., Bagnato V.S. A Multicenter Clinical Study of Expected and Unexpected Side Reactions During and After Skin Cancer Treatment by Photodynamic Therapy // Skinmed. – 2017. – Vol. 15(2). – P. 113-118.
25. Филоненко Е.В., Григорьевых Н.И, Иванова-Радкевич В.И. Фотодинамическая терапия больного базальноклеточным раком кожи ушной раковины стадии T3N0M0 (клиническое наблюдение) // Biomedical Photonics. – 2021. – Т. 10, № 4. – С. 68–70.
26. Paoli J., Gyllencreutz J.D., Fougelberg J., Backman E.J., Modin M., Polesie S., Zaar O. Nonsurgical Options for the Treatment of Basal Cell Carcinoma // Dermatol Pract Concept. – 2019. – Vol. 9(2). – P. 75-81.
27. Каплан М.А., Романко Ю.С. Фотодинамическая терапия как новый радикальный метод лечения у больных с рецидивными опухолями «неудобной» локализации // Вопросы онкологии. – 2000. – Т. 46, № 2. – С. 238.
28. Lucena S.R., Salazar N., Gracia-Cazaña T., Zamarrón A., González S., Juarranz Á., Gilaberte Y. Combined Treatments with Photodynamic Therapy for Non-Melanoma Skin Cancer // Int J Mol Sci. – 2015. – Vol. 16(10). – P. 25912-25933.
29. Ferrara F., Lacava R., Barisani A., Messori S., Patrizi A., Bardazzi F., Vaccari S. Combined CO2 laser and photodynamic therapy enhances the efficacy of treatment of basal cell carcinomas // J Dtsch Dermatol Ges. – 2019. – Vol. 17(12). – P. 1251-1256.
30. Li X., Tan L., Kou H., Zhang J., Wang Y., Li G., Lu Y. Ocular preservation through limited tumor excision combined with ALA-PDT in patients with periocular basal cell carcinoma // Photodiagnosis Photodyn Ther. – 2019. – Vol. 27. – P. 291-294.
31. Zeitouni N.C., Sunar U., Rohrbach D.J., Paquette A.D., Bellnier D.A., Shi Y., Wilding G., Foster T.H., Henderson B.W. A prospective study of pain control by a 2-step irradiance schedule during topical photodynamic therapy of nonmelanoma skin cancer// Dermatol Surg. – 2014. – Vol. 40(12). – P. 1390-1394.

CASE REPORTS

PHOTODYNAMIC THERAPY IN A PATIENT WITH HPV-ASSOCIATED LSIL OF THE CERVICE (CLINICAL CASE)

Filonenko E.V., Grigoryevykh N.I., Kaprin A.D.
P.A. Herzen Moscow Oncology Research Center – branch of FSBI NMRRC
of the Ministry of Health of the Russian Federation, Moscow, Russia

Abstract

The article describes a clinical example of timely, safe and effective photodynamic therapy (PDT) in a patient diagnosed with human papillomavirus (HPV) CIN I-associated cervical cancer after ineffective vaccination with 4-valent Gardasil vaccine. Clinical case demonstrates the low effectiveness of HPV vaccination in patients with established HPV infection. In this patient, the lack of adequate treatment for about 1.5 years led to the development of HPV associated CIN I of the cervix. Center for Laser and Photodynamic Diagnostics and Therapy of Tumors and MRI P.A. Herzen, the patient underwent a course of antiviral PDT with the achievement of complete regression of dysplasia and complete eradication of HPV viruses. The patient tolerated the treatment well, without complications. The period of relapse-free follow-up is 19 months.

Key words: cervical dysplasia, CIN, photodynamic therapy, photosensitizer, HPV infection.

For citations: Filonenko E.V., Grigoryevykh N.I., Kaprin A.D. Photodynamic therapy in a patient with HPV-associated LSIL of the cervice (clinical case), *Biomedical Photonics*, 2022, vol. 11, no. 3, pp. 40–44. doi: 10.24931/2413–9432–2022–11-3-40–44.

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

ФОТОДИНАМИЧЕСКАЯ ТЕРАПИЯ БОЛЬНОЙ С ВПЧ–АССОЦИИРОВАННОЙ LSIL ШЕЙКИ МАТКИ (КЛИНИЧЕСКОЕ НАБЛЮДЕНИЕ)

Е.В. Филоненко, Н.И. Григорьевых, А.Д. Каприн
«Московский научно-исследовательский онкологический институт им. П.А. Герцена
– филиал ФГБУ «Национальный медицинский исследовательский центр радиологии»
Министерства здравоохранения Российской Федерации, Москва, Россия

Резюме

В статье описывается клинический пример проведения своевременной, безопасной и эффективной фотодинамической терапии (ФДТ) у пациентки с диагнозом ассоциированной с вирусом папилломы человека (ВПЧ) CIN I шейки матки после неэффективной вакцинации 4-валентной вакциной гардасил. Клиническое наблюдение демонстрирует низкую эффективность вакцинации против ВПЧ больных с установленной ВПЧ инфекцией. У данной пациентки отсутствие адекватного лечения на протяжении около 1,5 лет привело к развитию ВПЧ ассоциированной CIN I шейки матки. В Центре лазерной и фотодинамической диагностики и терапии опухолей и МР МНИОИ им. П.А. Герцена пациентке проведен курс противовирусной ФДТ с достижением полной регрессии дисплазии и полной эрадикации вирусов ВПЧ. Лечение пациентка перенесла хорошо, без осложнений. Срок безрецидивного наблюдения – 19 мес.

Ключевые слова: дисплазия шейки матки, CIN, фотодинамическая терапия, фотосенсибилизатор, ВПЧ-инфекция.

Для цитирования: Филоненко Е.В., Григорьевых Н.И., Каприн А.Д. Фотодинамическая терапия больной с ВПЧ–ассоциированной LSIL шейки матки (клиническое наблюдение) // Biomedical Photonics. – 2022. – Т. 11, № 3. – С. 40–44. doi: 10.24931/2413–9432–2022–11-3-40–44.

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

Introduction

The role of human papillomavirus (HPV) persistence in the development of cervical cancer is now considered proven. For the prevention of cancer in these clinical situations, the currently accepted tactic is the use of the HPV vaccine. Clinical use of Gardasil was initiated in 2006, Cervarix in 2009. Since then, there has been a decrease in HPV infection in a young population of women aged 12-26 years who have been vaccinated, but prophylactic vaccination does not induce immunity in individuals with established HPV infection or neoplasms caused by HPV. The quadrivalent Gardasil vaccine is considered proven to be 99% effective against HPV and, as a result, is effective in preventing genital warts and genital warts caused by HPV types 6, 11, 16 and 18.

According to the literature, approximately 80% of sexually active women will contract some type of HPV infection during their lifetime, and in most cases this will be a transient, asymptomatic infection that is cured by the immune system within 6 months to two years. Only as a result of persistence of HPV infection can low or high grade cervical intraepithelial neoplasia develop, which can eventually progress to cervical cancer [1]. Given the above, timely and effective treatment of HPV infection is a technology for the prevention of cervical cancer.

We present a clinical case.

Clinical case

Patient N., aged 28, consulted a gynecologist in August 2019. Papillomavirus infection of the cervix (HPV) was diagnosed for the first time. PCR study for HPV revealed the presence of HPV types 31, 56 and 59 in the amount of 1.1 x 10 * 3 st.; 6.9 x 10 * 2 st.; 1.2 x 10 * 4 st., respectively. No treatment was given. During the re-examination on February 1, 2020, carriage of HPV type 31 was detected in the amount of 1.3 x 10 * 5 st., Type 59 – 6.9 x 10 * 5 st. HPV vaccination was recommended. From March to September 2020, the patient was vaccinated and twice revaccinated with the Gardasil-4 vaccine.

During the control PCR for HPV from 09/23/20, carriage of HPV type 31 was detected in the amount of 4.7 x 10 * 3 st., Type 56 – 8.6 x 10 * 5 st. Cytological examination for atypical cells on September 23, 2020 revealed ASC-US. The patient was left under dynamic observation. Cytological examination of smears from the cervix dated 12/04/20 revealed LSIL/CIN I, dyskaryosis, koilocytosis; PCR study for HPV – the presence of HPV type 31 in the amount of 4.1 x 10 * 3 st., Type 56 – 1.3 x 10 * 6 st.

The patient independently applied to the P.A. Herzen Moscow Oncology Research Center.

A cytological study was performed on December 17, 2020 No. 20/6-3009: a smear from the cervix – cells of the squamous epithelium of the intermediate and surface layers, in some of which there are signs of HPV infection, koilocytic transformation, metaplastic cells, elements of inflammation, moderate mixed flora – conclusion – cytogram of moderate inflammation, squamous cells with signs of HPV infection and low-grade intraepithelial lesions – LSIL (CIN I); No. 20/6-3010: smear from the cervical canal – squamous epithelial cells, some of which have signs of koilocytic transformation, metaplastic cells, groups and clusters of cylindrical epithelium cells, blood cells, mucus – conclusion: squamous epithelial cells with signs of HPV infection and low-grade intraepithelial lesion – LSIL (CIN I). Immunocytochemical study No. 20/8-124 dated December 23, 2020 was performed using p16 and Ki67 markers. Some of the squamous epithelial cells showed a positive moderate expression of the p16 protein. The Ki67 proliferative activity index in squamous epithelial cells is low – conclusion: ICC data, together with cytological data, correspond to the picture of HPV-associated low-grade intraepithelial lesion – LSIL.

When viewed during the initial consultation at the P.A. Herzen Moscow Oncology Research Center – the external genital organs are formed correctly, the external opening of the urethra is without pathology; examination in the mirrors: the vaginal mucosa is not changed, on the mucous membrane of the cervix there is an endocervical ectopia around the external os up to 1.2 cm wide; PV: the cervix is not compacted, the uterine body is of a normal shape and size, dense, mobile, painless, the appendages on the left and right are not defined, their area is painless, the arches are free, infiltrates are not detected in the parameters, the utero-rectal depression is free, the rectovaginal septum is intact.

An MRI of the pelvic organs dated 01/10/21 with a targeted examination of the cervix showed an endocervix of a heterogeneous MRI signal, 2 to 3 mm thick, no volumetric formations of the cervix were detected. The vaults of the vagina and parameters are intact.

Ultrasound of the pelvic organs dated 01/12/21 (on the 9th day of the cycle) showed that the bladder was not changed. The uterus is 47.4x28.3x42.5 mm in size, the myometrium is homogeneous, the expansion of the arcuate veins of the uterine body up to 2.6 mm is noted; the endometrium is 8.3 mm thick, echopositive, homogeneous, the contours are clear, even. The thickness of the median structures of the cervix is up to 12.3 mm (duplication), with single small anechoic single inclusions, moderately pronounced vascularization. The cervical canal was dilated up to 1.5 mm due to anechoic contents. At the level of the isthmus, there are a few intra- and subendocervical cysts up to 4.3 mm in size. The left ovary is 32x20x25 mm in size, with follicles up to 19x11.4x18 mm, the right one is 29.4x14x24 mm, with follicles up to 7 mm in size. A meager amount of free fluid in the small pelvis is determined.

Taking into account the long-term persistence of HPV (about 1.5 years), the ineffectiveness of previously used therapeutic approaches, a decision was made at the council to conduct PDT.

In February 2021, a PDT course was conducted (Fig.).

At the control examination in July 2021, the mucous membrane of the cervix is smooth, uniform in color, there are no foci of ectopia of the cervical epithelium, a control cytological study was performed No. , groups of cells of cylindrical epithelium, cytogram without features; in scraping from the cervix – cells of the squamous epithelium of the surface layers without signs of atypia.

During the control examination in October 2021, another control cytological examination No. 77790000094 scraping from the cervical canal – NILM was performed; № 77790000093 scraping from the cervix – NILM; PCR study for HPV No. 77790000092 – HPV was not detected.

During the control examination in September 2022 – scraping from the cervical canal and cervix – NILM, PCR study for HPV – HPV was not detected.

The patient has been observed for more than 19 months without recurrence of LSIL, with complete eradication of HPV.

Discussion

Currently, HPV is recognized worldwide as an etiological factor in the development of cervical cancer and precancerous lesions, whose DNA is found in 99.7% of cases [2]. Cervical cancer is the fourth most common cancer in women worldwide and the fourth leading cause of death among women [3]. There are HPV groups of "low risk" (type 6, 11, 42, 43), they are practically not detected in cervical cancer. The HPV group of "high risk", which is isolated from cervical squamous cell carcinoma, includes viruses 16, 18, 31, 33, 35, 39, 45, 50, 51, 56, 58, 64 and 68 types [4]. Although some HPV infections may spontaneously resolve due to the work of the own immune system, studies have shown that "high risk" HPV infection is the main trigger for the development of lesions of the cervix [5]. Therefore, for patients with persistent high-risk infection for more than one year, timely treatment should be undertaken to prevent malignant lesions of the cervix. For patients with low-grade cervical disease, prompt and aggressive treatment should be undertaken to promote negative HPV conversion and prevent further progression of cervical intraepithelial neoplasia (CIN).

The onset of CIN can be defined as a complex mechanism of uncontrolled cell division that may involve cellular changes and epigenetic factors such as HPV gene integration. In the presence of HPV infection, DNA mutates in cellular and other environmental conditions, which leads to the integration and triggering of mechanisms for the synthesis of viral DNA and host DNA. Therefore, viruses can elude the mechanisms of cellular and humoral immune defense, promote cell proliferation and inhibit apoptosis [6].

CIN includes a number of pathological changes such as abnormal cell proliferation, poor differentiation, nuclear anomalies, and increased mitosis in cervical epithelial cells upon HPV stimulation. CIN is closely related to and precedes cervical cancer (CC) [7].

Previously, it was customary to divide CIN into three degrees depending on the degree of tumor cell dysplasia, namely, CIN I – mild, CIN II – moderate and CIN III – severe dysplasia / cr in situ, which reflects the continuous pathological process of the occurrence and development of cervical cancer [8].

As part of the lower anogenital squamous epithelial terminology (LAST) standardization project, squamous intraepithelial lesions (SIL) have been used to rename HPV-associated squamous epithelial lesions of the lower genital tract, including the cervix, to low-grade squamous intraepithelial lesions, LSIL and high-grade squamous intraepithelial lesions – HSIL [9].

Squamous intraepithelial HSIL lesions without proper treatment have a high risk of progression to invasive cervical carcinoma. The choice of treatment method is determined individually depending on the prevalence of the process, age, and other factors. The main treatment options are cervical conization using laser, ultrasound, electro- and radiosurgical techniques, cryotherapy and photodynamic therapy [10]. With regard to the treatment

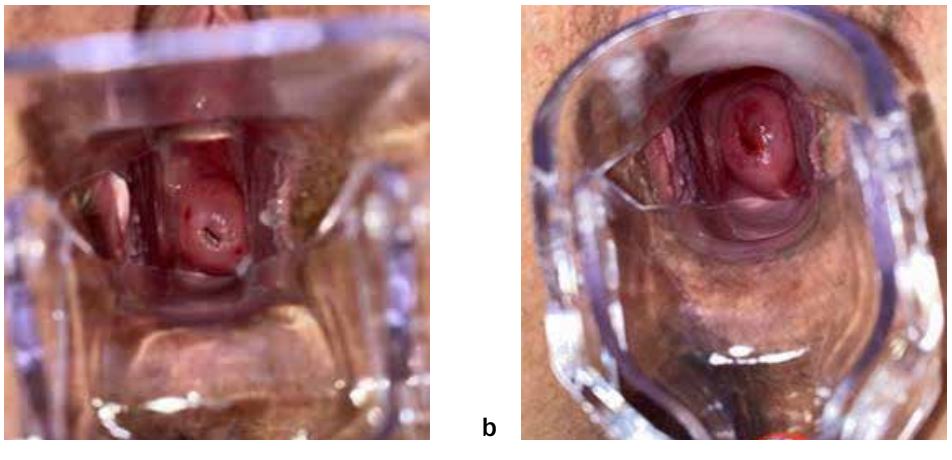


Рис. Фото шейки матки больной Н.: a – до ФДТ; b – 3 мес после ФДТ.
Fig. Photo of the cervix of the patient N.: a – before PDT; b – 3 months after PDT.

of LSIL, some experts believe that LSIL is only an instantaneous expression after HPV infection, which has different biological properties from malignant tumors with a high natural regression rate [11]. Rouzier R. et al. reported that 60-90% of LSILs can self-regress naturally within 2 years. While 30% of LSIL persist, at least 10% progress to HSIL [12].

Thus, early detection and treatment of persistent high-risk HPV infection and dysplasia can effectively prevent the onset of cervical cancer.

Thanks to the knowledge that HPV is the main etiological factor in the development of cervical cancer, measures have been developed to prevent cervical cancer – a prophylactic vaccine against HPV has been developed and has been used in clinical practice since 2006 [13].

In Russia, two types of vaccines are available that are used for vaccination against HPV: tetravalent, which should protect against infection with four types of HPV – 6, 11, 16 and 18 (Gardasil is produced by the Dutch pharmaceutical company MSD Gardasil Merck Sharp and Domu B.V.), and bivalent – from two types of HPV – 16 and 18 (Cervarix is produced in Belgium by GlaxoSmith-Kline Biologicals). In Europe, in addition to these two and 9-valent vaccines are used, which acts against nine types of HPV – 6, 11, 16, 18, 31, 33, 45, 52, 58, most often detected in cervical cancer after HPV 16 and 18 types [14]. However, even with cross-protection and an increase in the number of HPV types covered by the 9-valent vaccine, any HPV vaccine does not protect against all HPV types that increase the risk of cervical cancer [15].

In cases where the patient is diagnosed as a carrier of HPV, the use of the vaccine is not justified. In a number of countries, such as China, Russia, Mexico, Italy, and others, photodynamic therapy of HPV-associated precancer and initial cervical cancer is actively used. The mechanism of action is based on the ability of a number of drugs – photosensitizers (PS) to accumulate in tumor tissue and, when interacting with light radiation of a certain wavelength, initiate damage and / or destruction of tumor structures due to a series of photophysical processes. The main targets of photodynamic exposure are tumor cells, the microvascular network of the tumor and the surrounding stroma, and cellular elements of the body's immune system that infiltrate the tumor [16]. Along with the antitumor effect of treatment, PDT also has an antiviral effect due to the selective accumulation of PS in cells infected with HPV, followed by their direct phototoxic destruction.

The Medical Research Ethics Committee of the International Peace Maternity & Child Health Hospital of China Welfare Institute approved a study of 115 patients with HPV-associated CIN, patients admitted to the hospital from October 2020 to June 2021. The average age of patients was 35.71±2.51 years. Three courses of PDT were performed with an interval of 7-14 days with local application of the drug based on 5-ALA, followed by irradiation for 30 min with a power density of 80 mW/cm^2. The overall cure rate and negative HPV tests was 79.0%. The follow-up period for patients ranged from 3 to 6 months after treatment [17].

In another study, PDT was performed on 30 Mexican women in one of the states of Veracruz at the age of 33. Based on cytological analysis, molecular tests, and histopathological evaluation of biopsy specimens, patients were divided into two groups: CIN I with high-risk HPV and high-risk HPV without CIN. Three patients withdrew from the study due to pregnancy. PDT was performed with a topically applied preparation based on 5-ALA, followed by irradiation of the cervix with a light dose of 200 J/cm^2. Histological examination of biopsy samples taken before PDT and 3, 6, and 12 months after treatment was performed. In the group of women only infected with HPV, the following results of PDT were achieved: 73% of patients got rid of the infection according to PCR data 3 months after PDT, 6 months after PDT. the number of patients with complete eradication of HPV increased to 80%. When observed for 12 months treatment outcome was maintained. In the HPV + CIN I group, 3 months after PDT, 42% of 12 patients showed complete regression of CIN I, when followed up for 12 months – there were no recurrences. In addition, 75% of HPV-infected patients with CIN I got rid of the infection according to PCR smears taken after 3 months, after 6 months the number of cases of complete eradication of HPV increased to 83%, this percentage remained constant for 12 months. In cases where, in addition to HPV infection, bacterial vaginosis was diagnosed, it was eliminated in 83% of patients after 3 months after PDT, the absence of vaginosis persisted for all 12 months observations [18].

In our clinical observation, we present the data of observation and treatment of a patient who, against the background of long-term persistence of highly oncogenic HPV types 31 and 56, developed cervical dysplasia – LSIL. An attempt by doctors at the place of residence to vaccinate Gardasil-4 could not achieve a positive result in relation to already existing viruses. PDT resulted in HPV eradication and LSIL cure.

Conclusion

The clinical observation presented in the article demonstrates the low effectiveness of HPV vaccination in the presence of persistent HPV infection, as well as the possibility and expediency of using antiviral PDT in these clinical situations.

There is also information in the world literature on the effective use of PDT with 5-ALA for antiviral purposes in women with HPV persistence in the absence of atypia of the cervical epithelium.

Thus, antiviral PDT with 5-ALA is a promising technology that can lead to the eradication of HPV infection and, as a result, the prevention of cervical cancer.

REFERENCES

1. Berman T.A., Schiller J.T. Human papillomavirus in cervical cancer and oropharyngeal cancer: One cause, two diseases. *Cancer*, 2017, vol. 123, pp. 2219-2229. doi: 10.1002/cncr.30588.
2. Zur Hausen H., Boyle P., Waggoner S. Peng Guan et al. Human papillomavirus types in 115789 HPV-positive women: A meta-analysis from cervical infection to cancer, *Int J Cancer*, 2013, vol. 131(10), pp. 2349-2359. doi: 10.1002 / ijc.27485.
3. Olusola P., Banerjee H.N., Philley J.V., Dasgupta S. Human papilloma virus-associated cervical cancer and health disparities, *Cells*, 2019, vol. 8, p. 622.
4. Onuki M., Matsumoto K., Iwata T., Yamamoto K., Aoki Y. et al. Human papillomavirus genotype contribution to cervical cancer and precancer: implications for screening and vaccination in Japan, *Cancer Sci*, 2020, vol. 111, pp. 2546-2557.
5. Zheng J.J., Song J.H., Yu C.X., Wang F., et al. Difference in vaginal microecology, local immunity and HPV infection among childbearing-age women with different degrees of cervical lesions in Inner Mongolia, *BMC Womens Health*, 2019, vol. 19, pp. 109.
6. Chan C.K., Aimagambetova G., Ukybassova T., Kongrtay K., Azizan A. Human papillomavirus infection and cervical cancer: epidemiology, screening, and vaccination-review of current perspectives, *J Oncol*, 2019, vol. 2019 eCollection, p. 3257939. doi: 10.1155/2019/3257939.
7. Mitra A., Tzafetas M., Lyons D., Fotopoulou C., Paraskevaidis E., Kyrgiou M. Cervical intraepithelial neoplasia: screening and management, *Br J Hosp Med (Lond),* 2016, vol. 77, pp. 118-123.
8. Castle P.E., Murokora D., Perez C., Alvarez M., Quek S.C., Campbell C. Treatment of cervical intraepithelial lesions, *Int J Gynaecol Obstet*, 2017, vol. 138, pp. 20-25.
9. Darragh T.M., Colgan T.J., Cox J.T., Heller D.S., et al. Members of LAST Project Work Groups. The lower anogenital squamous terminology standardization project for HPV-associated lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology, *Arch Pathol Lab Med*, 2012, vol. 136, pp. 1266-1297.
10. Li X., Liu M., Ji Y., Qu P. The effectiveness of cold-knife conization (CKC) for post-menopausal women with cervical high-grade squamous intraepithelial lesion: a retrospective study, *BMC Surg*, 2021, vol. 21, p. 241.
11. Segura S.E., Ramos-Rivera G., Hakima L., Suhrland M., Khader S. Low-grade squamous intraepithelial lesion, cannot rule out high-grade lesion: diagnosis, histological outcomes and human papillomavirus results. *Cytopathology*, 2019, vol. 30, pp. 99-104.
12. Rouzier R. Management of CIN1, *J Gynecol Obstet Biol Reprod (Paris)*, 2008, vol. 37(S1), pp. 114-120.
13. Muñoz N., Kjaer S.K., Sigurdsson K., Iversen O.E., Hernandez-Avila M., et al. Impact of Human Papillomavirus (HPV)-6/11/16/18 Vaccine on All HPV-Associated Genital Diseases in Young Women, JNCI J. Natl. *Cancer Inst,* 2010, vol. 102, pp. 325-339. doi: 10.1093/jnci/djp534.
14. Chabeda A., Yanez R.J.R., Lamprecht R., Meyers A.E., Rybicki E.P., Hitzeroth I.I. Therapeutic vaccines for high-risk HPV-associated diseases, *Papillomavirus Res*, 2018, vol. 5, pp. 46-58. doi: 10.1016/j.pvr.2017.12.006.
15. Higgins L.M., Dirksing K.N., Ding L., Morrow C.D., Widdice L.A., Kahn J.A. Adolescents' intention and self-efficacy to follow Pap testing recommendations after receiving the HPV vaccine, *Hum. Vaccines Immunother*, 2016, vol. 12, pp. 1498-1503. doi: 10.1080/21645515.2016.1150395.
16. Filonenko E.V. Clinical implementation and scientific development of photodynamic therapy in Russia in 2010-2020, *Biomedical Photonics*, 2021, vol. 10(4), pp. 4-22. doi: 10.24931/2413-9432-2021-9-4-4-22 25.
17. Yi Chen, Ying Xu, Zhengrong Zhang, Zhenhong Xiong, and Dan Wu. 5-aminolevulinic acid-mediated photodynamic therapy effectively ameliorates HPV-infected cervical intraepithelial neoplasia, *Am J Transl Res*, 2022, vol. 14(4), pp. 2443-2451.
18. Maldonado A.E., Osorio Peralta M.O., Moreno V.A., Martínez Guzmán, L. A., et al. Effectiveness of Photodynamic Therapy in Elimination of HPV-16 and HPV-18 Associated with CIN I in Mexican Women, *Photochemistry and Photobiology*, 2017, vol. 93(5), pp. 1269-1275.doi:10.1111/php.12769

ЛИТЕРАТУРА

1. Berman T.A., Schiller J.T. Human papillomavirus in cervical cancer and oropharyngeal cancer: One cause, two diseases // Cancer. – 2017. – vol. 123. – P. 2219-2229. doi: 10.1002/cncr.30588.
2. Zur Hausen H., Boyle P., Waggoner S. Peng Guan et al. Human papillomavirus types in 115789 HPV-positive women: A meta-analysis from cervical infection to cancer // Int J Cancer. – 2013. – Vol. 131(10). – P. 2349-2359. doi: 10.1002 / ijc.27485.
3. Olusola P., Banerjee H.N., Philley J.V., Dasgupta S. Human papilloma virus-associated cervical cancer and health disparities // Cells. – 2019. – vol. 8. – P. 622.
4. Onuki M., Matsumoto K., Iwata T., Yamamoto K., Aoki Y. et al. Human papillomavirus genotype contribution to cervical cancer and precancer: implications for screening and vaccination in Japan // Cancer Sci. – 2020. – vol. 111. – P. 2546-2557.
5. Zheng J.J., Song J.H., Yu C.X., Wang F., et al. Difference in vaginal microecology, local immunity and HPV infection among childbearing-age women with different degrees of cervical lesions in Inner Mongolia // BMC Womens Health. – 2019. – vol. 19. – P. 109.
6. Chan C.K., Aimagambetova G., Ukybassova T., Kongrtay K., Azizan A. Human papillomavirus infection and cervical cancer: epidemiology, screening, and vaccination-review of current perspectives // J Oncol. – 2019. – vol. 2019 eCollection. – P. 3257939. doi: 10.1155/2019/3257939.
7. Mitra A., Tzafetas M., Lyons D., Fotopoulou C., Paraskevaidis E., Kyrgiou M. Cervical intraepithelial neoplasia: screening and management // Br J Hosp Med (Lond) . – 2016. – vol. 77. – P. 118-123.
8. Castle P.E., Murokora D., Perez C., Alvarez M., Quek S.C., Campbell C. Treatment of cervical intraepithelial lesions // Int J Gynaecol Obstet. – 2017. – vol. 138. – P. 20-25.
9. Darragh T.M., Colgan T.J., Cox J.T., Heller D.S., et al. Members of LAST Project Work Groups. The lower anogenital squamous terminology standardization project for HPV-associated lesions: background and consensus recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology // Arch Pathol Lab Med. – 2012. – vol. 136. – P. 1266-1297.
10. Li X., Liu M., Ji Y., Qu P. The effectiveness of cold-knife conization (CKC) for post-menopausal women with cervical high-grade squamous intraepithelial lesion: a retrospective study // BMC Surg. – 2021. – vol. 21. – P. 241.
11. Segura S.E., Ramos-Rivera G., Hakima L., Suhrland M., Khader S. Low-grade squamous intraepithelial lesion, cannot rule out high-grade lesion: diagnosis, histological outcomes and human papillomavirus results // Cytopathology. – 2019. – vol. 30. – P. 99-104.
12. Rouzier R. Management of CIN1 // J Gynecol Obstet Biol Reprod (Paris). – 2008. – vol. 37(S1). – P. 114-120.
13. Muñoz N., Kjaer S.K., Sigurdsson K., Iversen O.E., Hernandez-Avila M., et al. Impact of Human Papillomavirus (HPV)-6/11/16/18 Vaccine on All HPV-Associated Genital Diseases in Young Women. JNCI J // Natl. Cancer Inst. – 2010. – vol. 102. – P. 325-339. doi: 10.1093/jnci/djp534.
14. Chabeda A., Yanez R.J.R., Lamprecht R., Meyers A.E., Rybicki E.P., Hitzeroth I.I. Therapeutic vaccines for high-risk HPV-associated diseases // Papillomavirus Res. – 2018. – vol. 5. – P. 46-58. doi: 10.1016/j.pvr.2017.12.006.
15. Higgins L.M., Dirksing K.N., Ding L., Morrow C.D., Widdice L.A., Kahn J.A. Adolescents' intention and self-efficacy to follow Pap testing recommendations after receiving the HPV vaccine // Hum. Vaccines Immunother. – 2016. – vol. 12. – P. 1498-1503. doi: 10.1080/21645515.2016.1150395.
16. Филоненко Е.В. Клиническое внедрение и научное развитие фотодинамической терапии в России в 2010-2020 гг. // Biomedical Photonics. – 2021. – Т. 10, № 4. – С. 4-22. doi: 10.24931/2413-9432-2021-9-4-4-22
17. Yi Chen, Ying Xu, Zhengrong Zhang, Zhenhong Xiong, and Dan Wu. 5-aminolevulinic acid-mediated photodynamic therapy effectively ameliorates HPV-infected cervical intraepithelial neoplasia // Am J Transl Res. – 2022. – vol. 14(4). – P. 2443-2451.
18. Maldonado A.E., Osorio Peralta M.O., Moreno V.A., Martínez Guzmán, L. A., et al. Effectiveness of Photodynamic Therapy in Elimination of HPV-16 and HPV-18 Associated with CIN I in Mexican Women // Photochemistry and Photobiology. – 2017. – vol. 93(5). – P. 1269-1275.doi:10.1111/php.12769

ЖИВАЯ
ЭНЕРГИЯ
СВЕТА

Фоторан e6®

НОВЫЙ РОССИЙСКИЙ ПРЕПАРАТ ДЛЯ ФОТОДИНАМИЧЕСКОЙ ТЕРАПИИ

- Действующее вещество природного происхождения
- Отсутствие аллергических реакций
- Отсутствие гепато и нефротоксичности, низкая фототоксичность
- Быстрое накопление в патологической ткани – 1,5-3 часа
- Длительное хранение без потери активности вещества – 3 года
- Низкая стоимость

ПАРТНЁРЫ:

Национальный медицинский исследовательский центр онкологии

Адыгейский республиканский онкологический диспансер

СККОД

По вопросам приобретения

+7 (495) 659-64-93
+7 (499) 726-26-98

ФОТОДИТАЗИН®

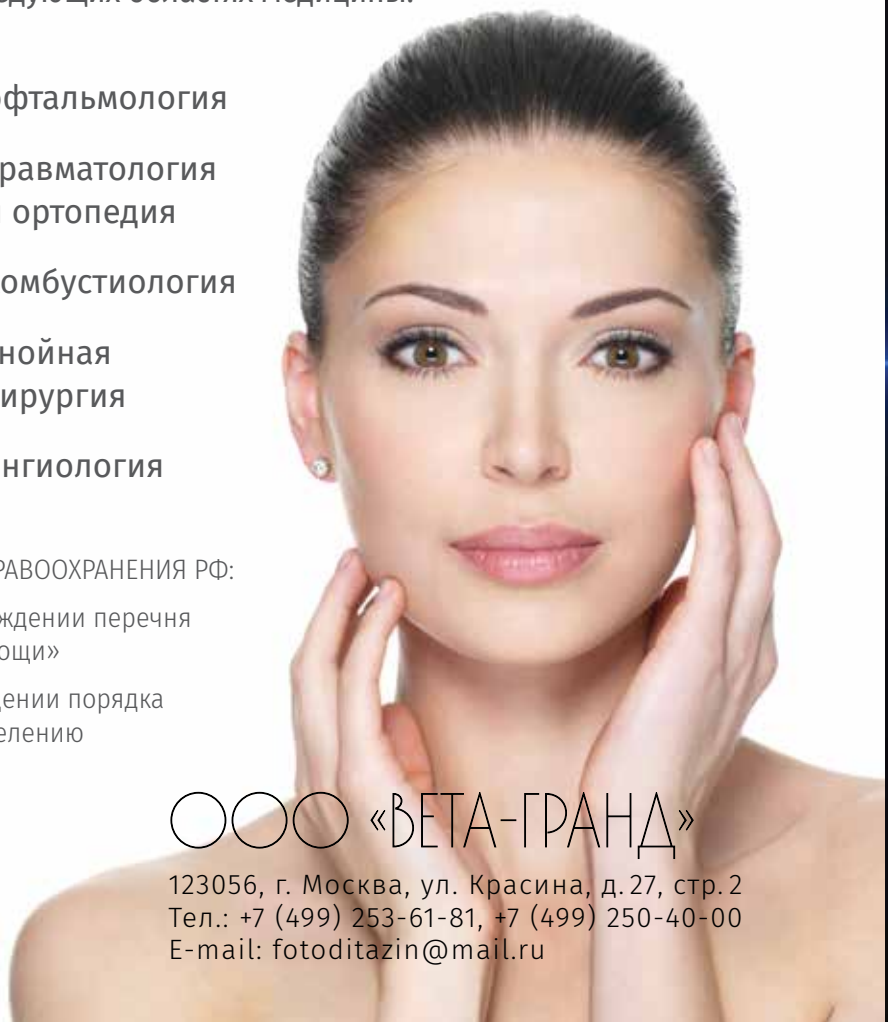
[fotoditazin]

фотосенсибилизатор хлоринового ряда

«ФОТОДИТАЗИН®» гель — РУ № ФСР 2012/13043 от 03.02.2012 г.
«ФОТОДИТАЗИН®» концентрат для приготовления раствора для инфузий — РУ № ЛС 001246 от 18.05.2012 г.

«ФОТОДИТАЗИН®» применяется для флюоресцентной диагностики и фотодинамической терапии онкологических заболеваний различных нозологических форм, а так же патологий не онкологического характера в следующих областях медицины:

- дерматология
- гинекология
- урология
- торакальная хирургия
- стоматология
- нейрохирургия
- офтальмология
- травматология и ортопедия
- комбустиология
- гнойная хирургия
- ангиология



В соответствии с приказами МИНИСТЕРСТВА ЗДРАВООХРАНЕНИЯ РФ:

Приказ № 1629н от 29 декабря 2012 г. «Об утверждении перечня видов высокотехнологичной медицинской помощи»

Приказ № 915н от 15 ноября 2012 г. «Об утверждении порядка оказания медицинской помощи взрослому населению по профилю „онкология“»

www.fotoditazin.com
www.фотодитазин.рф

ООО «ВЕТА-ГРАНД»
123056, г. Москва, ул. Красина, д. 27, стр. 2
Тел.: +7 (499) 253-61-81, +7 (499) 250-40-00
E-mail: fotoditazin@mail.ru