EFFECT OF NON-INVASIVE FRACTIONAL PHOTOTHERMOLYSIS ON THE EFFICACY OF TRANSDERMAL PHOTOSENSITIZATION IN THE EXPERIMENT *IN VIVO*

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Abstract

In an *in vivo* pilot study, the efficiency of noninvasive fractional laser photothermolysis (NFLP) as a transdermal system for application photosensitization of mouse skin before photodynamic therapy (PDT) was studied. For NFLP, a laser (λ = 970 nm) with an average power of 4 W and a pulse frequency of 50 Hz was used. An area of the skin of the anterior abdominal wall of mice was irradiated. After NFLP, a photosensitizer (PS) based on chlorin e6 in the form of a gel (0.5%) was applied to the skin with an application time of 30 min. Then, laser PDT (λ = 662 nm) was performed with a power of 2 W in a scanning pulse-periodic mode with a frequency of 5 Hz and a light spot area on the skin of 1.2 mm^2. The results of histological examination, confocal and electron microscopy showed the features of transdermal distribution of chlorin e6 after NFLP. PS fluoresces in all skin layers and the subcutaneous fat layer, indicating its deep penetration into the hypodermis after NFLP compared to conventional cutaneous application. The advantages of NFLP as a transport system for successful penetration of the gel form of chlorin e6 through all skin layers are demonstrated. Electron microscopy showed transdermal transport of PS in the form of nanosized microspheres and particles absorbed by macrophages and fibroblasts. It was also shown for the first time that pulsed PDT after NFLP leads to the formation of nanosized foci of photodestruction up to the border of the reticular layer of the skin and the hypodermis.

Keywords: photothermolysis, transdermal drug transport, photosensitisation, chlorin e6, photodynamic therapy, fluorescence, confocal microscopy, electron microscopy, light microscopy.

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ВЛИЯНИЕ НЕИНВАЗИВНОГО ФРАКЦИОННОГО ФОТОТЕРМОЛИЗА НА ЭФФЕКТИВНОСТЬ ТРАНСДЕРМАЛЬНОЙ ФОТОСЕНСИБИЛИЗАЦИИ И ФОТОДИНАМИЧЕСКОЙ ТЕРАПИИ В ЭКСПЕРИМЕНТЕ *IN VIVO*

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Резюме

В пилотном исследовании *in vivo* изучена эффективность неинвазивного фракционного лазерного фототермолиза (НФЛФ), как трансдермальной системы для аппликационной фотосенсибилизации кожи мышей перед фотодинамической терапией (ФДТ). Для НФЛФ использовали лазер (λ = 970 нм) со средней мощностью 4 Вт и частотой импульсов 50 Гц. Проводили облучение участка кожи перед-

ней брюшной стенки мышей. После НФЛФ на кожу наносили фотосенсибилизатор (ФС) на основе хлорина е6 в виде геля (0,5 %) с временем аппликации 30 мин. Затем проводили лазерную ФДТ (λ = 662 нм) с мощностью 2 Вт в сканирующем импульсно-периодическом режиме с частотой 5 Гц и площадью светового пятна на коже 1,2 мм^2. Результаты гистологического исследования, конфокальной и электронной микроскопии показали особенности трансдермального распределения хлорина е6 после проведения НФЛФ. ФС флуоресцирует во всех слоях кожи и подкожно-жировом слое, что указывает на его глубокое проникновение в гиподерму после НФЛФ по сравнению с обычной накожной аппликацией. Продемонстрированы преимущества НФЛФ как транспортной системы для успешного проникновения гелевой формы хлорина е6 через все слои кожи. Электронная микроскопия показала трансдермальный транспорт ФС в виде наноразмерных микросфер и частиц, поглощаемых макрофагами и фибробластами. Также было впервые показано, что импульсная ФДТ после НФЛФ приводит к формированию наноразмерных очагов фотодеструкции вплоть до границы сетчатого слоя кожи и гиподермы.

Ключевые слова: фототермолиз, трансдермальный транспорт лекарств, фотосенсибилизация, хлорин е6, фотодинамическая терапия, флуоресценция, конфокальная микроскопия, электронная микроскопия, световая микроскопия.

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Introduction

Photodynamic therapy (PDT) is a modern method of treating malignant neoplasms and precancerous conditions based on systemic and local administration of a photosensitizer (PS) and exposure to visible electromagnetic radiation in the presence of tissue oxygen. PS accumulates in pathological tissues and, under the influence of light of a certain wavelength, converts tissue oxygen into active oxygen species (ROS), which destroy pathologically altered cells (precancerous pathology, malignant neoplasms), bacteria and viruses [1-3].

The various effects of PDT include cell necrosis and apoptosis, vascular thrombosis, activation of the immune system and immunosuppression, stimulation of collagenogenesis, as well as antimicrobial and antiviral action. Owing to the development of laser and endoscopic equipment and the emergence of various PS, the range of PDT applications has expanded significantly in such areas as oncology, dermatology, cosmetology, gynecology, otolaryngology, dentistry, urology, ophthalmology and even the treatment of the new coronavirus infection [4-8].

The main goal of PDT of non-oncological diseases, in contrast to the treatment of malignant tumors, is the modulation of cellular functions by exposure to low-power light energy and low-dose PDT with anti-inflammatory, antimicrobial and reparative purposes. However, at present, there are no standardized protocols for PDT of non-oncological diseases [9, 10].

Over the past thirty years, three generations of PS have been developed for clinical use. Chlorin e6, which belongs to the second generation, is capable of generating ROS when irradiated with red light of 662 nm. Due to the hydrophobicity of chlorin e6 molecules, it can penetrate the peptide glycan layers of the walls of gram-positive bacteria, providing successful antimicrobial PDT, including against Mycobacterium tuberculosis [11-15].

Methods of local photosensitization, such as intradermal injection or application of gel forms of the drug, allow the accumulation of PS only in the case of a superficial location of the pathological lesion or for the purpose of reparative regeneration of the skin [16]. This makes PDT more targeted and reduces the risk of phototoxicity by reducing the dose of PS. However, deep penetration of PS is limited by the protective functions of the skin, which requires the development of new transdermal delivery systems (TDS) [17].

As an alternative, a new non-invasive version of fractional laser photothermolysis (FLP) is proposed as a TDS for skin photosensitization before the PDT procedure [18].

With NFLP, lasers with a wavelength of less than 2000 nm are used, emitting energy in pulses that leave the epidermis intact and, forming microthermal zones without destruction of the dermis, cause the synthesis of new collagen [19, 20].

Further efforts to develop minimally invasive phototherapeutic techniques with increased efficacy have led to the study of options combining FLP techniques with pulsed light therapy or with PDT with 5-aminolevulinic acid (5-ALA) [21].

Since the NFLP technology with a wavelength of λ = 970 nm has not previously been studied in relation to skin photosensitization with chlorin e6 for subsequent PDT procedures, we initiated an *in vivo* study of the features of the intradermal distribution of chlorin e6 when locally applied to healthy skin after NFLP.

Materials and Methods

Photosensitizer

The drug radachlorin (Rc) in the dosage form "RadaGel" (OOO "Radapharma", Moscow, Russia) containing sodium salt of chlorin e6, obtained from the chlorophyll of the alga Spirulina Plantensis using a patented method, was used as a photosensitizer.

Experiment. Animals

To study the intradermal distribution of Rc after NFLP, male HTAAAKR mice (SPF-vivarium nursery of the ICG SB RAS (Federal Research Center, Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia) aged 3 months (n = 16) were used.

The studies were carried out in accordance with the requirements of Directive 2010/63/EU of the European Parliament and of the Council of the European Union on the protection of animals used for scientific purposes. The animals were kept on a standard diet with unlimited access to water and food. The experimental study protocol was approved at a meeting of the local ethics committee of the Research Institute of Clinical and Experimental Lymphology - branch of the ICG SB RAS on 08.07.2019 (protocol No. 151).

The animals were fixed in a supine position. A 1×1 cm area was selected on the shaved skin of the anterior abdominal wall of the animals, on which NFLP was performed using semiconductor laser "Lakhta Milon" (OOO "Kvalitek", Moscow, Russia) (λ = 970 nm) with an average power of 4 W in a scanning pulse-periodic mode with a pulse duration of 10 ms at a frequency of 50 Hz (t = 10 ms, f = 50 Hz) through a quartz-polymer light guide with a focusing lens (OOO "Polironic", Moscow, Russia). The diameter of the light spot at the focus was 1.2 mm, the energy dose density in the pulse was 25 J/cm^2. Fractional laser exposure to the skin lasted 30 s.

Rc in a concentration of 0.5% was applied with a sterile spatula at a dose of 0.1 g per 1 cm^2 of the selected skin area. The exposure of the drug on the skin was 30 min, after which its remains were removed with a sterile napkin.

PDT was performed with a "Lakhta Milon" semiconductor laser (OOO "Kvalitek", Moscow, Russia) (λ = 662 nm) and a power of 2 W in a scanning pulse-periodic mode with a laser pulse duration of 100 ms at a frequency of 5 Hz (t = 100 ms, f = 5 Hz) through an optical fiber with a lens focusing a light spot of 0.002 cm^2 on the skin, providing a laser energy density of 40 J/cm^2 on the tissue surface.

The animals were divided into 4 groups. Animals in the 1st group (n = 4) underwent NFLP of the abdominal wall skin. In the 2nd group (n = 4), similar preparation of the skin with NFLF was performed and Rc was applied. After 30 min, the remnants of PS were removed with a sterile napkin and PDT was performed. In the 3rd group (n = 4), Rc was applied to the skin of the animals for 30 min, then its remnants were removed with a napkin and PDT was performed. In the 4th group (n = 4), the shaved skin of the animals was not exposed to the effects.

After completion of the effects, euthanasia was performed by dislocation of the cervical vertebrae, then the anterior abdominal wall was excised and skin samples were prepared for histological examination, confocal and electron microscopy. The material was fixed in a 4% paraformaldehyde solution.

Confocal microscopy

Confocal microscopy was performed on cryosections of skin tissue. To prepare cryosections, skin samples were fixed with a 4% paraformaldehyde solution for 1 day, then washed with a cooled sodium phosphate buffer (PBS) solution and immersed in a 30% sucrose solution for 1 day. After that, the studied tissues were placed in foil with a TissueTek medium and frozen at -70°C.

Sections from frozen tissue were obtained on an HM 550OP cryostat (Zeiss, Germany) and prepared taking into account their transverse orientation for subsequent assessment of the depth of penetration of the PS. Images were obtained on a laser scanning microscope LSM 780 NLO AxioObserver Z1 (Zeiss, Germany) in the Collective Use Center for Microscopic Analysis of Biological Objects of the Institute of Cytology and Genetics SB RAS.

To analyze the fluorescence of PS in skin samples, confocal microscopy was performed with fluorescence excitation at a wavelength of λ = 458 nm. Fluorescence was recorded at a wavelength of λ = 675 nm. The images obtained by confocal microscopy were processed using the Zeiss Efficient Navigation (ZEN) 2010 microscope software. The recorded fluorescence values of the preparation were expressed in optical units (r.u.).

For each animal, the fluorescence value of optical sections in each skin layer was calculated using the microscope software. After that, the data for each individual were aggregated and calculations were performed.

Light and electron microscopy

To assess the structure of the epidermis, papillary and reticular layers of the skin, samples were prepared by fixing in a 4% paraformaldehyde solution prepared on Hanks' medium, then fixed for 1 hour in a 1% osmium tetroxide (OsO_4) solution on a phosphate buffer (pH = 7.4), dehydrated in ethyl alcohol of increasing concentration and embedded in Epon. Semi-thin sections of 1 μm thickness were obtained on a Leica EM UC7 ultramicrotome (Leica Microsystems, Germany), stained with toluidine blue and examined using a LEICA DME light microscope (Leica Microsystems, Germany). Digital micrographs were obtained using the AVerTV6 computer program.

For examination of skin samples in an electron microscope, ultrathin sections of 70-100 nm thickness were obtained using a Leica EM UC7 ultramicrotome (Leica Microsystems, Germany) and stained with uranyl acetate and lead citrate. Digital photographs of skin fragments were obtained at a magnification of ×30,000 with JEM 1400 electron microscope (JEOL, Tokyo, Japan) and analyzed using ImageJ software (National Institutes of Health, Bethesda, MD, USA). Microscopic analysis was

performed at the Multidisciplinary Center for Microscopy of Biological Objects at the Institute of Cytology and Genetics SB RAS (Novosibirsk, Russia).

Morphometry

Morphometric analysis of digital photographs of ultrathin skin sections (25 fields of view in each group at a magnification of x15,000) was performed using Image J software (Wayne Rasband, USA). The sizes of Rc in the cytoplasm of epithelial cells, fibroblasts and in the interstitium were determined.

Statistical processing

Since the study was preliminary and the number of samples in each group was 4 units, which does not allow making confident conclusions about the presence of observed patterns in the population regardless of the choice between parametric and nonparametric criteria, in order to indicate the direction for further work, it was decided to use sample means and standard deviations as descriptive statistics (and, accordingly, parametric criteria for intergroup comparisons), since it seems that for a given number of objects, such statistics will be a more visual and informative characteristic of the sample than the mode, median and interquartile range. The calculation of the average values of fluorescence intensity and standard deviation (μ, σ) in each group was performed using the Statistica v.10 program (Statsoft, USA). Pairwise comparisons of groups were performed using the Student's criterion.

Separately, it should be notet that we received an insignificant number of observations, and these results justify the need to conduct a study with a larger number of objects.

Statistical analysis of the data obtained when determining the size of Rc particles was performed using the Statistica v.10 computer program (Statsoft, USA). The Kolmogorov–Smirnov statistical test was used to check the variation series for normality. The results of the ultrastructural analysis did not meet the criteria for normal distribution, so the description of the quantitative characteristics is presented as a median and the first and third quartiles – Me (Q1; Q3).

Results

Confocal Microscopy

During confocal microscopy of skin samples from group 4 (control) and from group 1 after NFLP, we did not detect any obvious tissue fluorescence (Fig. 1A, B), and the numerical values of fluorescence intensity correspond to weak autofluorescence of endogenous chromophores, with the exception of all epidermis samples after NFLP, which were deprived of the ability to fluoresce due to photobleaching under the influence of pulsed infrared laser radiation of 970 nm (Table).

In groups 2 and 3, pronounced fluorescence was noted, which in group 2 was much more intense than in group 3 (Fig. 1). Evaluating the distribution of Rc in the skin layers by fluorescence intensity in numerical values of r.u., it was possible to determine that the maximum fluorescence in the skin samples in group 3 reached 2500 r.u. in the epidermal layer and sharply decreased to 600 r.u. at the border of the reticular layer.

Micrographs of skin samples in group 2, obtained after NFLP with subsequent application photosensitization of Rc, demonstrate an increase in the transdermal distribution of PS, which is confirmed by fluorescence in all layers of the skin with the highest intensity in the epidermal and papillary layers.

Thus, in the epidermal layer, the maximum possible fluorescence intensity value for recording by a microscope was recorded at 4095 r.u., which turned out to be 1.7 times greater than in group 3 with conventional application of PS. At the border of the epidermal and reticular layers, the fluorescence intensity reached 2000 r.u., which exceeded the maximum values in the third group by 3.3 times.

It should be noted that after NFLP with application photosensitization, deeper penetration of Rc into the hypodermis occurs, where fluorescence up to 500 r.u. is preserved, also spreading in the subcutaneous fat layer (Fig. 1D).

This fact confirms the property of non-invasive pulsed laser energy at a wavelength of 970 nm, focused on the epidermis, to increase the transport of chlorine series PS through all layers of the skin in 30 minutes of application exposure of the drug.

The aggregate data on the comparative quantitative characteristics of the fluorescence intensity of the skin

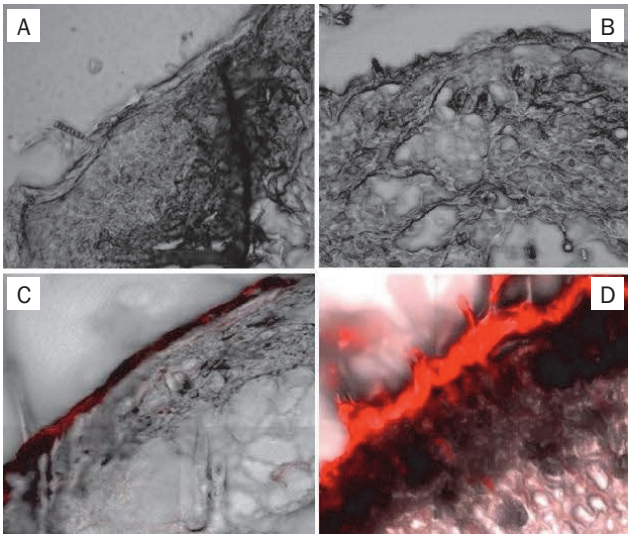

Рис. 1. Конфокальная микроскопия образцов кожи: A – контроль (группа 4); B – НФЛФ (группа 1); C – ФС+ФДТ (группа 3); D – НФЛФ+ФС+ФДТ (группа 2).
Fig. 1. Confocal microscopy of skin samples: A – control (group 4);. B – NFLP (group 1); C – PS +PDT (group 3); D – NFLP+PS+PDT (group 2).

Таблица
Интенсивность флуоресценции слоёв кожи исследуемых групп
Table
Fluorescence intensity of skin layers in the studied groups

Группы Groups	Воздействие Impact	Интенсивность флуоресценции Fluorescence intensity			
		Эпидермис + сосочковый слой (о.е.) Epidermis + papillary layer (o.u.) μ,σ	Сетчатый слой (о.е.) The mesh layer (o.u.) μ,σ	Гиподерма (о.е.) Hypoderma (o.u.) μ,σ	Все слои (о.е.) All layers (o.u.) μ,σ
1 (n = 4)	НФЛФ NFLP	0	2,74; 1,32	3,13; 1,77	2,8; 1,06
2 (n = 4)	НФЛФ + ФС + ФДТ NFLP+PS+PDT	3487,56; 150,64*	140,22; 16,48*	16,03; 2,78	240,35; 26,45*
3 (n = 4)	ФС + ФДТ PS+PDT	633,38; 92,31*	22,68; 4,77*	9,76; 1,75	41,44; 6,15*
4 (n = 4)	Контроль Control	7,36; 6,23	0,98; 0,61	0	1,5; 0,69

*p < 0,05

layers of the studied groups are presented in the form of a table. At $p < 0.05$, significant differences from the control group were noted.

Comparison of the results of skin photosensitization demonstrates obvious advantages of preliminary NFLP, which creates conditions for the penetration of chlorin e6 through all layers of the dermis to the subcutaneous fat layer, which was confirmed by an increase in the fluorescence intensity of PS in all layers by 5.8 times, in the papillary layer by 5 times, in the reticular layer by 6 times (table).

Light Microscopy

Light microscopy examination of skin samples allowed to register the peculiarity of structural changes in the compared groups that occur under the influence of non-invasive laser energy pulses focused on the stratum corneum.

Comparing the control samples from group 4 with the samples from group 1, we were convinced that after NFLP there were no thermal damages and the differentiation and cellular composition of the epidermis were preserved, but after non-invasive laser exposure there was a compaction of the papillary layer of the skin and edema of the reticular layer (Fig. 2B).

In group 3, after pulsed PDT, there was a significant compaction of the papillary and reticular layers with a violation of their differentiation (Fig. 2C).

Exposure of the skin to NFLP followed by pulsed PDT in the second group led to compaction of the reticular layer of the skin while maintaining the differentiation and cellular composition of the epidermis (Fig. 2D).

Thus, according to light microscopy data, the presented variants of laser energy effects, devoid of damaging effects on the skin, have a number of different effects, represented by compaction of only the papillary

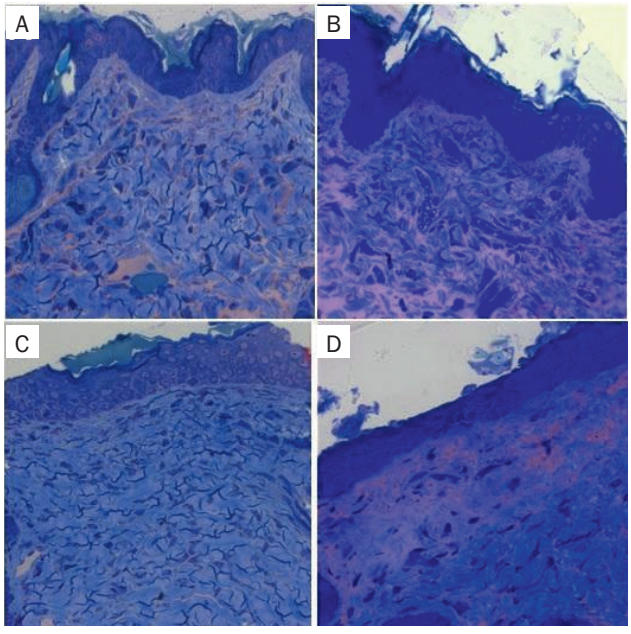

Рис. 2. Световая микроскопия (увеличение x400, окраска толуидиновым синим): A – контроль (группа 4); B – после НФЛФ (группа 1); C – после ФДТ+ФС (группа 3); D – после НФЛФ+ФС+ФДТ (группа 2).
Fig. 2. Light microscopy (magnification x400, stained with toluidine blue): A – control (group 4); B – after NFLP (group 1); C – after PDT+PS (group 3); D – after NFLP +PS+PDT (group 2).

layer after NFLP, deeper compaction of the papillary and reticular layers after PDT, and the deepest compaction of the reticular layer after a combination of NFLP with subsequent PDT.

Electron microscopy

Electron microscopy allowed to characterize the changes in the skin after non-invasive laser treatment at the ultrastructural level, to see the features of the extra- and intracellular distribution of Rc in the skin structures, and to confirm the passage of laser energy through all layers of the skin by the photodynamic reactions that took place.

In group 1, after NFLP without the use of PS, we noted the compaction of epithelial cells, the papillary layer and structures located on the border of the papillary and reticular layers of the skin (Fig. 3C, D).

In animals of group 3, after cutaneous application of Rc, its uneven distribution in the skin layers was recorded in the form of spherical particles with an average size of 536.0 (436.0; 668.5) nm. The maximum accumulation of the drug occurred in the epithelial layer (Fig. 4A).

When assessing the effects of pulsed PDT in group 3, a relatively low density of formation of foci of photodestruction in the epithelium and papillary layer of the dermis was noted with a high density of Rc particles absorbed by epithelial cells.

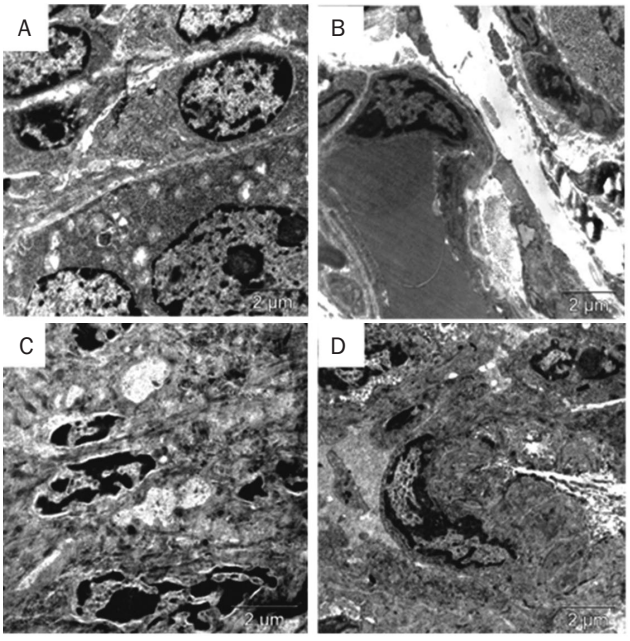

Рис. 3. Ультраструктурная организация кожи животных в контроле и после НФЛФ, электронная микроскопия: A-B – контроль (группа 4); A – ультраструктура эпителия; B – структура сосочкового слоя дермы; C-D – после НФЛФ (группа 2); повышение плотности эпителия (C) и сосочкового слоя дермы (D).
Fig. 3. Ultrastructural organization of animal skin in control and after NFLP. Electron microscopy: A-B – control (group 4); A – ultrastructure of the epithelium; B – structure of the papillary dermis; C-D – after NFLP (group 2); increased density of the epithelium (C) and papillary dermis (D).

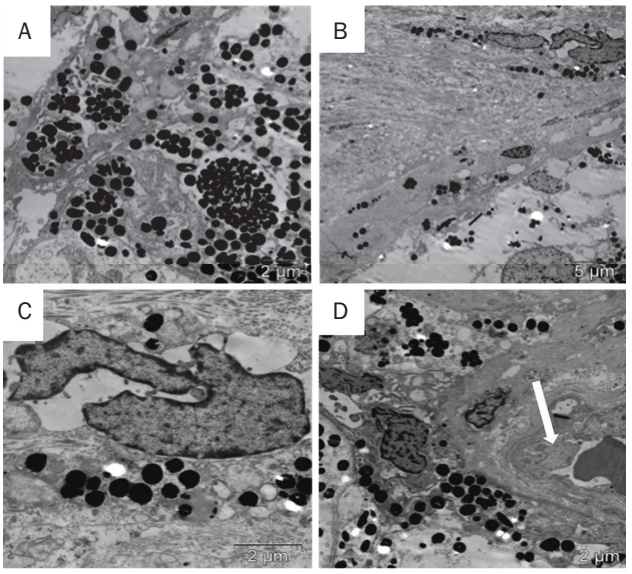

Рис. 4. Ультраструктурная организация кожи после аппликационной фотосенсибилизации ФС и последующей импульсной ФДТ (группа 3): A – накопление в эпителии массы интактных частиц ФС; B – наличие интактных частиц ФС и разрозненных очагов фотодеструкции; C – накопление ФС и очагов фотодеструкции в цитоплазме макрофага; D – частицы ФС находятся в сосочковом слое дермы и отсутствуют в цитоплазме эндотелия кровеносного сосуда (стрелка).
Fig. 4. Ultrastructural organization of the skin after application photosensitization with PS and subsequent pulsed PDT (Group 3): A – accumulation of a mass of intact PS; B – the presence of intact sl PS particles and isolated foci of photodestruction; C – PS and foci of photodestruction in the cytoplasm of the macrophage; D – PS particles are located in the papillary layer of the dermis and are absent in the cytoplasm of the endothelium of the blood vessel (arrow).

We believe that in this group of animals that did not receive preliminary NFLP, photodynamic effects are realized to a lesser extent due to the shielding of the energy of laser pulses absorbed by skin pigments and excess PS on the stratum corneum of the epidermis.

This assumption was confirmed after studying the results of electron microscopy of skin samples from group 2 mice after NFLP with application photosensitization and pulsed PDT.

We found that after NFLP, successful accumulation of Rc particles occurs in epithelial cells, and the subsequent photodynamic reaction is represented by a significant number of scattered vesicle-like foci of photodestruction with an average size of 681.0 (174.5; 1485.0) nm (Fig. 5A).

Similar in size and spherical shape, multiple foci of photodestruction were recorded in the interstitium of not only the papillary layer, but also in the reticular layer of the dermis (Fig. 5B, C).

The obtained data indicate that the preparation of the skin with NFLP significantly improves the penetration of Rc in the form of spherical microaggregates into all layers

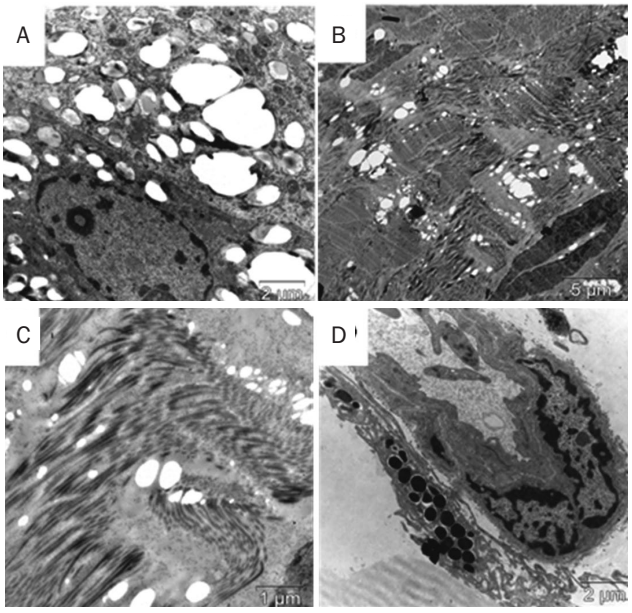

Рис. 5. Электронная микроскопия. Структура кожи в первый день после НФЛФ, аппликационной фотосенсибилизации ФС и импульсной ФДТ (группа 2): А – эпителий с множественными везикулоподобными очагами фотодеструкций; В и С – интерстиций сетчатого слоя дермы содержит скопления сферических очагов фотодеструкций; D – накопление частиц интактных микросфер ФС в цитоплазме фибробласта.
Fig. 5. Electron microscopy. Skin structure on the first day after NFLP, application photosensitization of PS and pulsed PDT (group 2): A – epithelium with multiple areas of photodamage, B and C – the interstitium of the reticular layer of the dermis contains clusters of spherical foci of photodestruction. D – accumulation of particles of intact PS microspheres in the cytoplasm of fibroblasts.

of the dermis, reaching the cytoplasm of the fibroblasts of the reticular layer at the border with the subcutaneous fat (Fig. 5D).

The effect of formation of multiple foci of photodestruction in the depth of the reticular layer in the hypodermis, characteristic only of cases from group 2 (Fig. 5C), indicates an increase in the efficiency of PDT after NFLP due to weakening of the epidermal shielding and compaction of the reticular layer (Fig. 2B, D), improving the light transmission of the skin, and an increase in the energy density of light pulses to levels that excite PS molecules to the triplet state in deeper layers of the skin.

Therefore, after NFLP, the energy of photons in the PDT process penetrates significantly deeper and, consequently, increases the medicinal activity of Rc in group 2 with the initially identical applied dose of PS in the compared groups 2 and 3.

An important fact is that we have recorded that by the beginning of the PDT procedure, PS does not move into the vascular sector of the intact skin and for this reason, microthrombosis of vessels, characteristic of PDT of tumors, is prevented (Fig. 4D).

Discussion

Local application of PS is associated with limitations due to the barrier function of the skin, especially the stratum corneum, which prevents their absorption. When PS is applied to healthy skin, the depth of its penetration is limited by the stratum corneum and does not exceed 12 ± 5 μm during 1 hour of cutaneous exposure [22].

In the search for optimal transdermal systems, non-ablative photothermolysis with a 1550 nm laser was successfully tested to increase the penetration of 5-ALA in PDT of acne [21]. As studies have shown, non-ablative laser techniques, devoid of invasiveness, minimally damage the stratum corneum [19,20,23].

In our study, we sought to find out whether non-invasive photothermolysis improves the penetration of PS into the deep layers of the skin and increases the effectiveness of PDT. The experiment was aimed at creating a multimodal effect on the skin using laser energy that improved light transmission and transdermal transport of PS with chlorin e6.

We chose laser radiation at a wavelength of 970 nm, focused on the stratum corneum with a pulse frequency of 50 Hz. This allowed to achieve partial damage to the stratum corneum, which weakened the reflection of energy during PDT and caused photobleaching of chromophores in the skin. The NFLP parameters ensured energy delivery through the lens, concentrating the power of 4 W in the focus on the epidermis without tissue coagulation, which led to photobiomodulation effects with activation of macrophages and fibroblasts for phagocytosis and transport of Rc granules.

The results showed that preliminary exposure to NFLP actually increases the depth of PS penetration in healthy skin without significant damage. Unlike traditional ablative FLP (AFLP), NFLP causes only point microtraumas, activating phagocytic cells that capture PS and transport it to the hypodermis.

The energy of the infrared laser pulse for traditional AFLP is such that it creates a column of damaged tissues to a certain depth, while the use of NFLP through a focusing lens leads only to the formation of a point light spot on the stratum corneum of the epidermis with subsequent scattering of the light beam in the deeper parts of the skin, which eliminates thermal damage.

Probably, pulsed laser action focused on the stratum corneum and epidermis, absorbed by skin pigments and hemoglobin in superficial capillaries and hair follicles, causes point microtraumas and secondary activation of phagocytic cells, which capture PS and actively transport it down to the hypodermis. The study is devoted to improving skin permeability using NFLP at a wavelength of $\lambda = 970$ nm for photosensitization in PDT.

Experiments on mice revealed features of Rc distribution when locally applied to skin prepared with NFLP. Although NFLP does not damage the epidermis,

confocal microscopy showed a loss of autofluorescence by the epidermis. This may indicate a photobleaching reaction of endogenous chromophores and an increase in the light transmission of the compacted epithelium.

Electron microscopy confirmed the increase in light transmission in the foci of photodestruction in the deep layers of the dermis revealed after PDT. Confocal microscopy showed an increase in the penetration of Rc into all layers of the dermis after the preparation of NFLP with an increase in its fluorescence intensity by 5.8 times. Electron microscopy demonstrated intradermal transport of Rc in the form of microspheres and their absorption by macrophages and fibroblasts, which is important for successful PDT.

Conclusion

We assume that NFLP activates the migration of phagocytic cells to the damaged area, which facilitates the transport of PS into the deep layers of the skin. Despite the demonstration of a new TDS for successful skin photosensitization, our study was not powerful enough to study the features of delayed skin reactions to pulsed PDT in the long-term period, which requires additional study.

Preliminary results of the pilot study emphasize the importance of studying tissue reactions to laser exposure at the micro level, which will open up new prospects for the use of NFLP as a transdermal system for drug delivery. We expect that the study will be useful for further development of PDT methods.

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