ORIGINAL ARTICLES

PHOTODYNAMIC EFFECTIVENESS OF LASER DIODE COMBINED WITH OZONE TO REDUCE STAPHYLICOCCUS AUREUS BIOFILM WITH EXOGENOUS CHLOROPHYLL OF DRACAENA ANGUSTIFOLIA LEAVES

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Abstract

Photodynamic inactivation is an effective treatment that uses light irradiation, photosensitizer and oxygen. The aim of this study was to determine photodynamic effectiveness of laser diode combined with ozone to reduce *Staphylococcus aureus* biofilm using exogenous chlorophyll (Chlo). The chlorophyll was extracted from leave of *Dracaena angustifolia*. To determine the antibacterial effect of *S. aureus* biofilm treatments, samples were separated into Chlo, Laser, Chlo+Laser, Ozone, Ozone+Laser, Chlo+Ozone+Laser categories. The data were analyzed using ANOVA test. The result of this study showed that Chlo+Ozone+Laser combine treatment at 20 s exposure of ozone with 4 min of irradiation time lead to 80.26 % reduction of biofilm activity, which was the highest efficacy of all the treatment groups. The combination of laser, chlorophyll and lower ozone concentration increases the effectiveness of photodynamic inactivation.

Keywords: antibacterial photodynamic therapy, laser irradiation, ozone, chlorophyll, staphylococcus aureus, biofilm.

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ЭФФЕКТИВНОСТЬ ФОТОДИНАМИЧЕСКОГО ВОЗДЕЙСТВИЯ В СОЧЕТАНИИ С ОЗОНОМ И ХЛОРОФИЛЛОМ ИЗ ЛИСТЬЕВ DRACAENA ANGUSTIFOLIA НА БИОПЛЕНКИ STAPHYLICOCCUS AUREUS

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Резюме

Фотодинамическая терапия – эффективный метод инактивации бактериальных биопленок, основанный на сочетании воздействий светового излучения, фотосенсибилизатора и кислорода. Цель данного исследования – определение эффективности лазерного облучения в сочетании с озоном при добавлении экзогенного хлорофилла для инактивации биопленки *Staphylococcus aureus (S. aureus)*. Хлорофилл был извлечен из листьев растения *Dracaena angustifolia*. В ходе исследования на образцах биопленки *S. aureus* оценивалась антибактериальная активность каждого фактора в отдельности (лазерное излучение, озон, хлорофилл) и нескольких их сочетаний (хлорофилл + лазерное облучение; озон + лазерное облучение; хлорофилл + озон + лазерное облучение). Полученные данные были проанализированы с использованием теста ANOVA. Анализ результатов исследования показал, что комбинированная обработка озоном в течение 20 с в присутствии хлорофилла с последующим облучением в течение 4 мин снизила активность биопленок на 80,26%, показав самую высокую эффективность среди всех тестируемых групп. Для повышения эффективности фотодинамической терапии бактериальных биопленок рекомендуется использовать комбинацию лазерного излучения с хлорофиллом и озоном.

Ключевые слова: антибактериальная фотодинамическая терапия, лазерное облучение, озон, хлорофилл, золотистый стафилококк, биопленка.

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Introduction

Biofilms are colonies of bacteria that produce a protective matrix layer called extracellular polymeric substance (EPS) and have higher virulence, resistance, and pathogenic properties [1–2]. Almost all antibiotics fail to control the biofilms [3–4]. In addition, there is a possibility of biofilm having the ability to reduce production of hydrogen peroxide (H_2O_2) which is a precursor of toxic molecules when DNA-protein synthesis changes [5]. Photodynamic inactivation (PDI) was investigated as an alternative method to reduce biofilms [6].

PDI is a therapy method using light, photosensitizer and oxygen. The PDI mechanism starts from the absorption of light the wavelength of which corresponds to the absorbance of photosensitizer. It can produce reactive oxygen species (ROS) through type I and II photochemical processes [7]. A previous report related of PDI using silver nanoparticles as photosensitizer and laser diode with an output of 450.00 ± 22.34 nm and 53.16 ± 0.01 mW. This combination could decrease the surviving biofilm compared to the laser diode itself by 64.48 ± 0.07% against 7.07 ± 0.23% at 6.13 ± 0.002 J/cm^2, respectively [8].

Street et al. reported that biofilm reduction depends on the energy dose of light absorbed by the photosensitizer [9]. One of such photosensitizers is chlorophyll. The advantage of using chlorophyll in PDT is that it is cheap, easy to obtain and has short incubation time [10]. Chlorophyll sensitizers are currently used in targeting cancer cells, microbes and infection [11-13].

The amount of ROS production could be increased by the presence of H_2O_2 at the target location during the photochemical process with photosensitizer or ozone delivery. Currently, the amount of ozone needed for treatment is still unclear because each tissue structure has specific properties such as periodontitis in dermatology [14]. Hegge et al. reported that the combination of ozone and PDI provides a high efficacy depending on the ozone concentration [15]. This study aimed to determine photodynamic effectiveness of laser diode combined with ozone to reduce *Staphylococcus aureus* biofilm using exogenous chlorophyll. The chlorophyll was extracted from *Dracaena angustifolia* leaves.

Materials and Methods

Biofilm and Crystal Violet Assay

The bacterial strain, *S. aureus* ATCC 25923 was inoculated from Tryptone Soy Agar (Oxoid, UK) and taken on Tryptone Soy Broth sterile (Oxoid, UK). The culture of bacteria were incubated at 37°C until bacterial colonies reached ~10^8 CFU/mL or 1.0 McFarland Standard. 100µL of bacteria culture was placed on 96-well microplates and 20 µL of 2% sucrose (w/v) were added. The samples were shaken at 36 rpm for 4 hours and incubated for 48 hours.

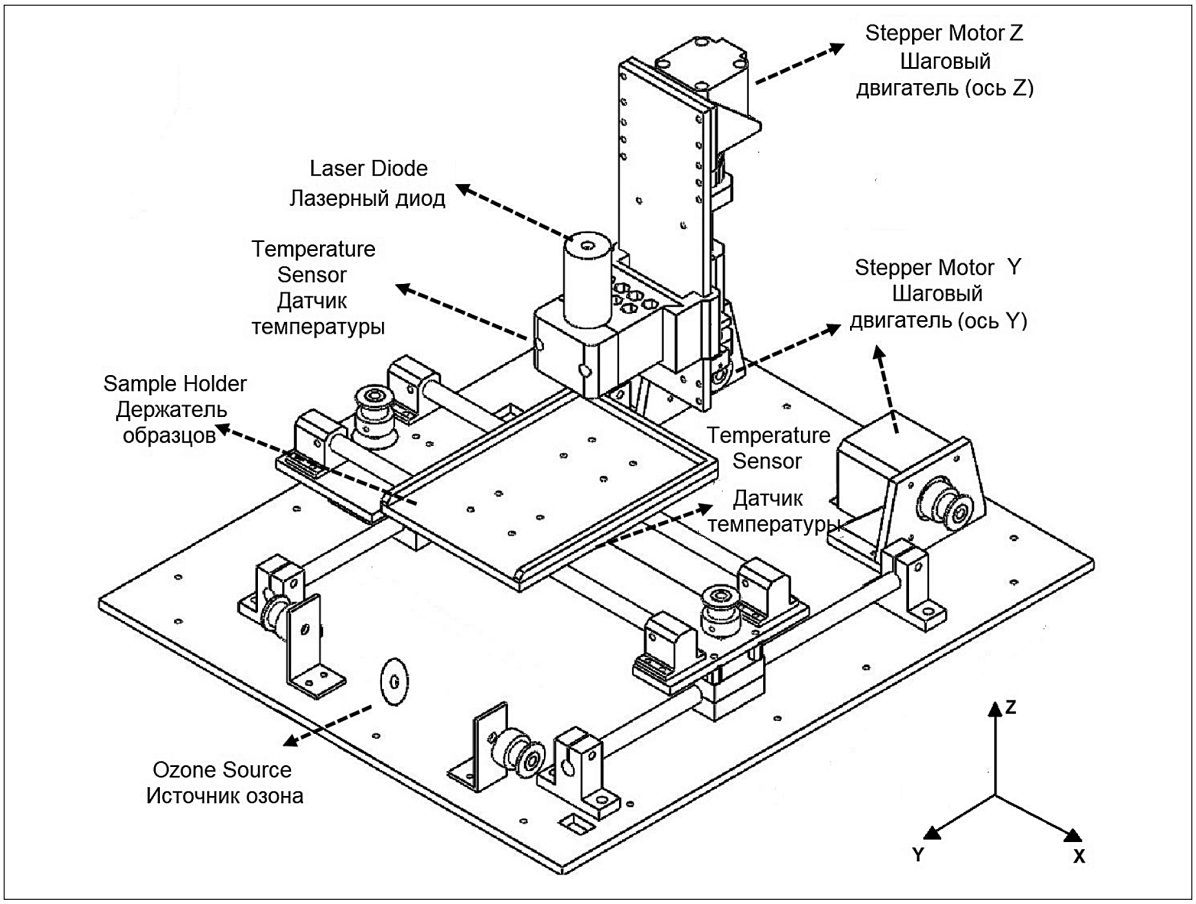
Biofilms were grown on well plate and rinses with Phosphate Buffered Saline (PBS) (pH 7.0) three times to remove individual cells. 100µL of 1% crystal violet were added to the sample and incubated with for 30 minutes. The samples were rinsed and dried for 3 hours. 50 µL 33% GAA (w/v) were added to the samples; the Optical Density (OD) value was measured at 595 nm.

Chlorophyll Extraction

The Chlorophyll (Chlo) was extracted from *D. angustifolia* leaves. 30 g of fresh leaves were added to 150 mL of 96% acetone and mashed into a pulp. The slice of leaves was filtered, purified, homogenized and precipitated, then put into the freezer at 20°C for 24 hours then filtered until it became a yellowish sediment. The stored filtrate was evaporated at 40°C. 60 g of silica gel was added to the filtrate. 8 g of silica gel and 16 ml of petroleum ether (PE) was then added and stirred homogenously for 5 minutes and then let to rest for 2 hours. The chlorophyll extract was dissolved in diethyl ether, then 5 g of silica gel was added and dried until a green crystal-like powder was formed. The dry chlorophyll extract was added to petroleum ether until it produced yellow filtrate. Finally, 96% acetone was added into the mixture until a clear silica gel color was produced. To analyze the characterization of chlorophyll, a thin layer chromatography and UV-Vis spectrophotometry (Bio-Rad) were used [15, 16].

Light Sources Apparatus for light irradiation

Fig. 1*a* shows an apparatus set-up of light and ozone source and Fig. 1*b* – a microcontroller block diagram.



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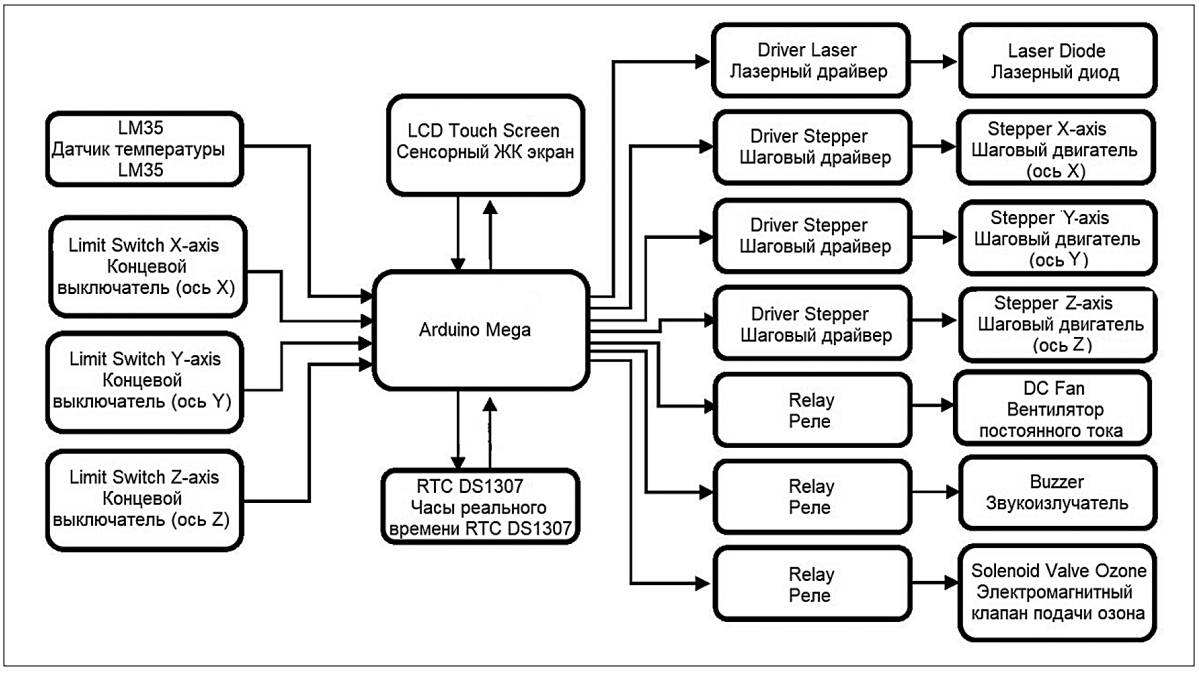

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Fig. 1. Apparatus set-up of light and ozone source *(a)* and microcontroller block diagram *(b)*
Рис. 1. Схема источника света и озона *(a)* и блоковая диаграмма микроконтроллера *(b)*

Table 1
Design of sample treatments to reduce S. aureus biofilm
Таблица 1
Типы воздействия на образцы с целью уменьшения биопленок S. aureus

Sample Treatments Воздействие на образцы	Chlorophyll Хлорофилл		Ozone Озон		Light Source Источник света	
	Volume (μL) Объем (мкл)	Concentration (ppm) Концентрация (ppm)	Exposure time (s) Время воздействия (с)	Concentration (mg/L) Концентрация (мг/л)	Irradiation Time (min) Время облучения (мин)	Energy Density (J/cm²) Плотность энергии (Дж/см²)
Chlo Хлорофилл	20	1.6	–	–	–	–
Ozone Озон	–	–	20	$3x10^{-3}$	–	–
	–	–	40	$5x10^{-3}$	–	–
	–	–	60	$1x10^{-2}$	–	–
Laser Лазер	–	–	–	–	1	4.35
	–	–	–	–	2	8.69
	–	–	–	–	3	13.04
	–	–	–	–	4	17.39
	–	–	–	–	5	21.74

The apparatus consists of a controller and laser module. The laser diode was previously characterized as having $\lambda = 399.81 \pm 15.11$ nm and $P_{out} = 30.43$ mW. The control box consists of microcontroller and LCD for setting the constant output intensity and time duration of the laser and controlling the position of the sample holder. The laser module consists of laser diode and sample holders.

All parameters were controlled as shown in Fig. 1*b*. The position of laser diode was controlled according of 96-well microplate position. The experimental treatment was performed in the dark room at ~27˚C.

Ozone Sources

According to the manufacturer, the ozone source has the output of 400 mg/hours. The time duration of ozone output was controlled and displayed on the LCD. The ozonising probe was aimed directly at the sample. The measurement of O_3 molecule concentration added on each time exposure was carried out using the iodometric titration method.

Experimental Design

Treatment were divided into the following groups: Chlo, Laser, Chlo+Laser, Ozone, Ozone+Laser, Chlo+Ozone+Laser, as shown in Table 1. The concentration of the chlorophyll (1.6 ppm) was based on its toxicity level. According to the described experimental groups, 0.1 ml of the *S. aureus* suspension was added to each well of sterile 96-well flat-bottom microtiter plates with lids. After the biofilm grew, 20 μl of the chlorophyll was added to the samples, which were then exposed to ozone and irradiated by laser diode at varying exposure time. Irradiation was done at a distance of 1 cm apart in a completely dark room. The growth of bacteria in the culture was monitored by measuring OD at 595 nm.

Statistical Analysis

The data measured in OD was converted to log CFU/mL by using Mc. Farland standard diagram with an equation of (log CFU/ml) = 3.771+12.374 x (OD). The biofilm reduction was measured using equation 1 [12].

$$\%\,Biofilm\,reduction = \frac{\left(\sum control - \sum treatment\right)}{\sum control} x100\% \quad (1)$$

For each treatment, the biofilm reduction percentage was calculated based on the control group with the untreated biofilm of *S. aureus*. The results of biofilm reduction were analyzed statistically by ANOVA test with a significance value of p = 0.05 using IBM SPSS Statistics Version 21.

Results

Characterization of Chlorophyll sensitizer

The result of pigment compounds of *D. angustifolia* leaves using thin layer chromatography was shown in Table 2. The Retention factor (R_f) is useful for finding out pigment compound during separation of pigment. The best ratio of the solvent system to get a form of pigments (without beta-carotene) on *D. angustifolia* leaves was petroleum ether and 96% acetone (8:2), respectively. The R_f has a valid value ranging from 0.2 to 1 which shows molecular polarity. Pigments of Chlorophyll-α obtained from *D. angustifolia* leaves extract showed 0.24 R_f value thus had low polarity.

Table 2
Result of thin layer chromatography of D. angustifolia leaves extract
Таблица 2
Результат тонкослойной хроматографии экстракта листьев D. Angustifolia

Fraction (color) Фракция (цвет)	Retention factor (R_f) Фактор удерживания (R_f)				Pigments Пигменты
	Solvent PE*: acetone Растворитель PE*:ацетон (8:2)	Solvent PE*: acetone Растворитель PE*:ацетон (9:1)	Solvent PE*: acetone Растворитель PE*:ацетон (8.5:1.5)	Solvent PE*: acetone Растворитель PE*:ацетон (8:2)	
Yellow Желтый	1	–	–	–	Beta-carotene Бета-каротин
Gray Серый	0.6039	0.1625	0.4875	0.6041	Pheophytin a Феофитин a
Yellow brown Желто-коричневый	0.4950	0.0875	0.3125	0.50	Pheophytin b Феофитин b
Blue green Сине-зеленый	0.2476	–	0.15	0.2375	Chlorophyll a Хлорофилл a
Yellow green Желто-зеленый	0.1818	–	0.0875	0.175	Chlorophyll b Хлорофилл b

* Petroleum ether
* Петролейный эфир

Based on Fig. 2, three Gaussian-like peaks with wavelengths of 414 nm, 458 nm, and 670 nm were obtained from the absorption spectrum of the *D. angustifolia* leaves extract. The maximum absorption is at 414 nm with FWHM of 40.40 ± 5.27 nm. This result would be used to determine the light source.

Efficacy of Treatment

The experimental results of the laser group are shown in Fig. 3. The comparison between the control group and chlo group had no significant difference. The Laser group at 2 min of irradiation time showed 13.06 log CFU/ml or 57.19% biofilm reduction while the Chlo+Laser group at 3 min showed about 12.30 log CFU/ml or 59.69 % biofilm reduction. However, the statistical analysis also showed there was no significant difference between both irradiation times indicated by the significance value of $p > 0.05$.

Treatment with the Ozone+Laser group (Fig. 4) showed that ozone exposure provides higher biofilm reduction efficacy within 40 s of exposure time. If laser irradiation was applied, they show the same pattern for each time of irradiation. The ozone group compared with the Ozone+Laser group at 40 s ozone exposure had significant difference with 20 and 60 s. 40 s of ozone treatment and 4 minutes of laser exposure produced around 11.98 log CFU/mL or 60.75% biofilm reduction.

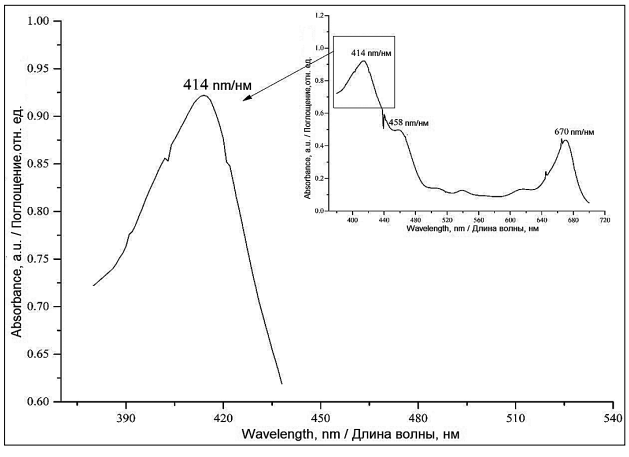

Fig. 2. The absorption spectrum of the *Dracaena angustifolia leaves* extract
Рис. 2. Спектр поглощения экстракта листьев *Dracaena angustifolia*

The result of the Ozone treatment combined with Laser and chlorophyll was shown in Fig. 5. Chlo+Ozone group had no significant difference at any exposure time. The Ozone and Chlo+Ozone treatment groups also had no significant difference. The Chlo+Ozone+Laser group at 20 s exposure to ozone with 4 min of irradiation time gave 6.02 log CFU/mL or 80.26% biofilm reduction and that was the highest biofilm reduction efficacy of all treatment groups.

Discussions

In photosynthesis, chlorophyll is an important pigment. Chlorophyll-α directly harvests light and transfers energy to reaction center on the photosynthetic system. The solvent system of chlorophyll extraction is an important factor in obtaining the separated fraction of pigment group. This study used petroleum ether (PE) and 96% acetone as the solvent system for chlorophyll extraction [17]. Retention factor value ranged from 0.2

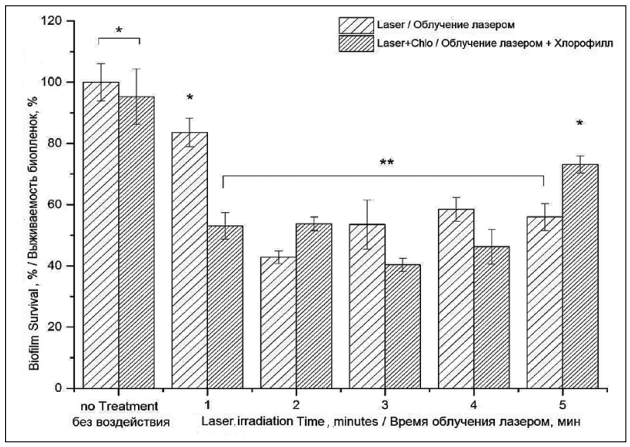

Fig. 3. The experimental results of the Laser group. Symbols * and ** show the significance level of p<0.05 and p>0.05, respectively
Рис. 3. Экспериментальные результаты по группе образцов с лазерным воздействием. Символы * и ** указывают на уровень значимости p<0,05 и p>0,05, соответственно

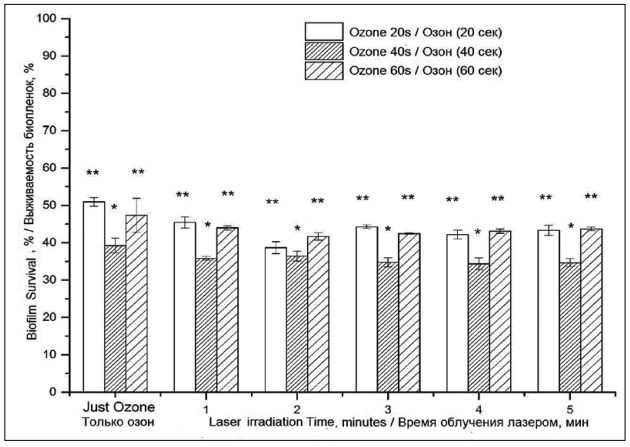

Fig. 4. Treatment results in the ozone and Laser groups. Symbol * and ** show the significance level of p<0.05 and p>0.05, respectively
Рис. 4. Результаты в группах образцов с воздействием озоном и лазером. Символы * и ** указывают на уровень значимости p<0,05 и p>0,05, соответственно

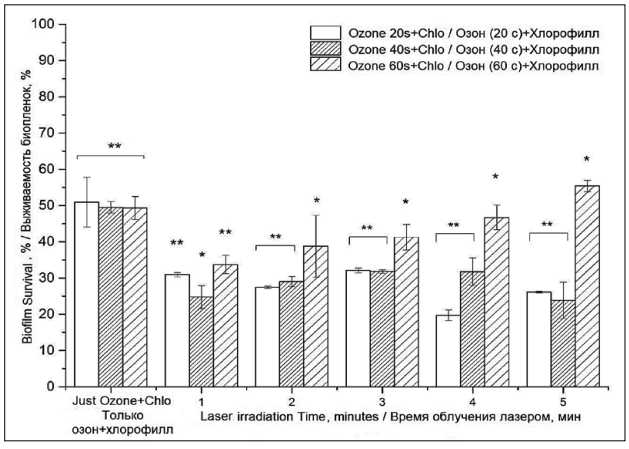

Fig. 5. The result of the Ozone treatment to be applied with Laser the addition of chlorophyll. Symbol * and ** showed the significance level of p<0.05 and p>0.05, respectively
Рис. 5. Результаты в группах образцов под воздействием озоном и лазером с добавлением хлорофилла. Символы * и ** указывают на уровень значимости p<0,05 и p>0,05, соответственно

to 1. PE and acetone (8:2) solvent had R_f of chlorophyll-a 0.25, but these contained beta-carotene pigment or carotenoid derivatives. The pigment acts as a protector against damage caused by ROS formation so that chlorophyll extraction had to be made without beta-carotene pigment. The absorbance spectrum of chlorophyll always has three peaks [18]. Chlorophyll extract had an absorption maximum at 414 nm, well correlated with peak wavelength of light source at $\lambda = 399.81 \pm 15.11$ nm.

The interaction of light and photosensitizer can produce toxic molecules. The photosensitizer in the body has an absorption spectrum that could be elucidated through an optical window that has optical absorption and scattering properties [19–20]. One of the endogen photosensitizers is porphyrin which has various types and absorbance spectra [21]. In previous study, the porphyrin derivatives of *S. aureus* bacteria have a wide peak absorption spectrum and maximum wavelength in blue region [13, 22]. The other study resulted in the PDT provide an increase in biofilm reduction with the addition of chlorophyll. The dose of light exposure, certain photosensitizer and oxygen play an important role in the success of the PDI [9, 23].

The combination of 3 treatments with high exposure to ozone should lead to reduction in survival. However, we finally realized that higher ozone concentration would increase generation of H_2O_2, whereas an organism (including animal, plant, and microbe) contains a protein that degrades H_2O_2 to oxygen and water. Based on the reaction (Table 3), hydrogen peroxide can bind with other hydrogen peroxide and form non-toxic molecules. Mishra explains that some hydrogen peroxide changes into non-toxic molecules before reaching the cell [24]. So, the point to increasing biofilm reduction is in the density of laser diode, chlorophyll and lower ozone concentrations.

Based on Table 3, the mechanism of Chlo+Laser group could generate of ROS (type II) and free radicals (type I). Photochemical type I reaction occurs when radical anions or cations are formed due to the transfer of electrons (or protons) to oxygen molecules and produce ROS because they easily react with the molecular oxygen. Photochemical type II reaction occurrs when energy is transferred to molecular oxygen to form singlet oxygen [19]. The dominant process depends on the chemical structure and the behavior of photosensitizers.

Treatment with ozone at 40 s exposure time caused greater reduction of biofilm because H_2O_2 are formed [26]. H_2O_2 molecules is an oxidant molecule that strengthens immunity by generating free radicals. The generation of free radicals obtained from the oxidative process of electron transfer of H_2O_2 through Fenton reaction. Ozone does not penetrate into the tissues but could spread to the cytoplasm [14].

Bocci said that ozone can react and polyunsaturated fatty acids (PUFA), antioxidants, thiol (-SH) compounds,

Table 3
The mechanisms PDI

Таблица 3
Механизмы фотодинамической инактивации

	Reactions Реакции	Ref. Ссылка
Ozone Озон	$R\text{-}CH{=}CH\text{-}R' + O_3 + H_2O \rightarrow R\text{-}CH{=}O + R'\text{-}CH{=}O + H_2O_2$	[14]
Photophysics Фотофизика	$PS + h\nu \rightarrow {}^1PS^*$ ${}^1PS^* \rightarrow {}^3PS^*$	[25]
Photochemical Type I Фотохимическая реакция Тип I	${}^3PS^* + {}^3PS^* \rightarrow PS^{+\cdot} + PS^{-\cdot}$ $PS^{-\cdot} + {}^3O_2 \rightarrow PS + {}^{\cdot-}O_2$	[19]
Photochemical Type II Фотохимическая реакция Тип I	${}^3PS^* + {}^3O_2 \rightarrow PS + {}^1O_2$	
Generation of ozone Генерация озона	${}^{\cdot-}O_2 + {}^{\cdot-}O_2 + 2H^+ \rightarrow {}^1O_2 + H_2O_2$ $H_2O_2 + {}^{\cdot-}O_2 \rightarrow {}^1O_2 + HO^{\cdot} + OH^-$ ${}^1O_2 + H_2O \rightarrow H_2O_3 \xrightarrow{{}^1O_2} H_2O_2 + O_3$	[26]
Catalase Каталаза	$H_2O_2 + H_2O_2 \rightarrow O_2 + 2H_2O$	[24]

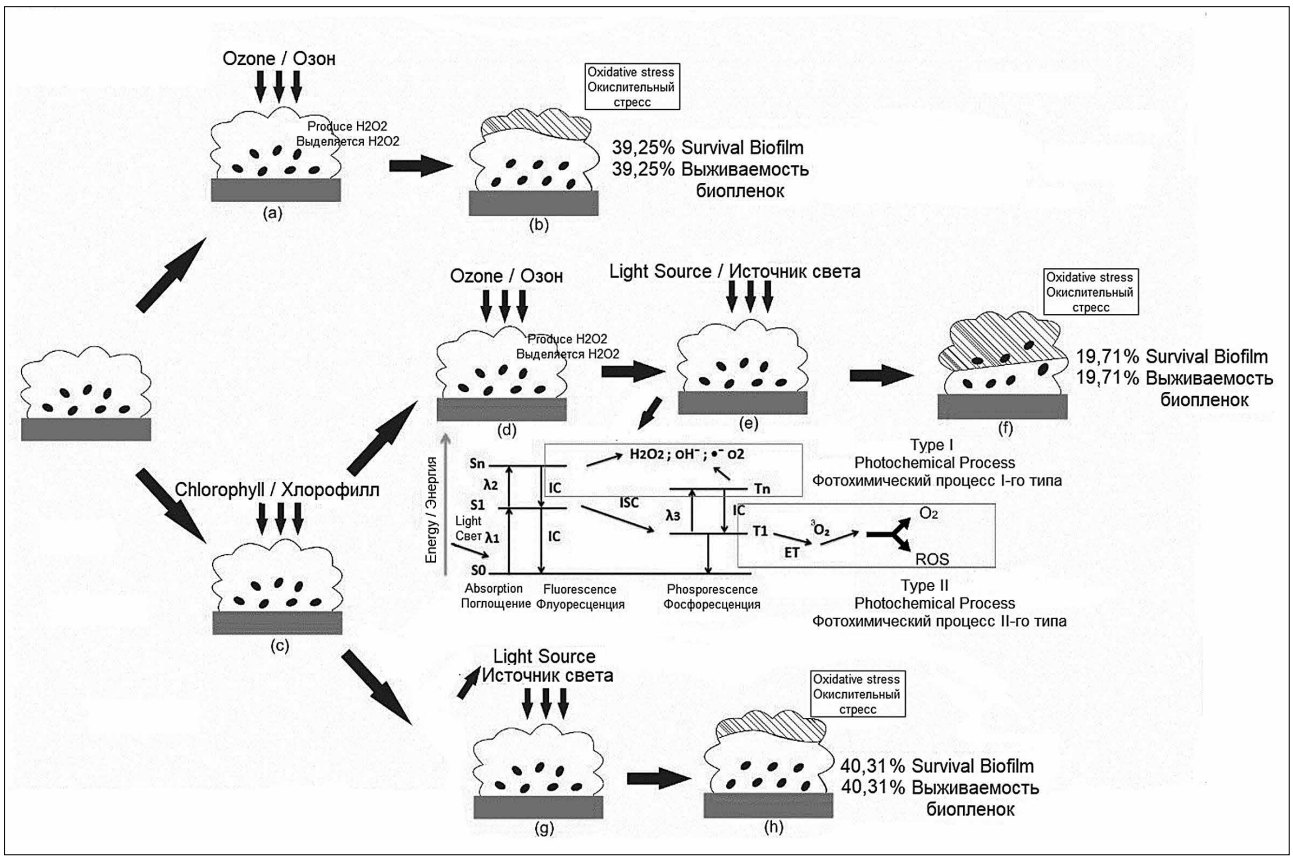

Fig. 6. The mechanisms of PDI treatment: (a) Ozone produces H_2O_2 in the biofilm surrounding that diffuses into the cytoplasm; (c) Samples with chlorophyll separated into 2 treatments; (d) Ozone produces a toxic molecule (H_2O_2); (e) and (g) Laser treatment could destroy EPS and increase the amount of toxic molecules through photochemical reaction.
Note: IC: internal conversion, ISC: intersystem crossing, Sn: singlet states, Tn:triplet states, ET: energy transfer; (b), (f), (h) the toxic molecule causes oxidative stress in biofilm

Рис. 6. Механизмы фотодинамической инактивации: (a) Озон производит H_2O_2 в окружении биопленки, который диффундирует в цитоплазму; (c) Образцы с хлорофиллом разделены в 2 типа воздействия; (d) Озон производит токсичную молекулу (H_2O_2); (e) и (g) лазерное воздействие может уничтожить внеклеточный матрикс (EPS) и привести к росту количества токсичных молекул за счет фотохимической реакции.
Примечание: IC: внутренняя конверсия, ISC: межсистемный переход, Sn: синглетные состояния, Tn: триплетные состояния, ET: перенос энергии; (b), (f), (h) токсичная молекула вызывает окислительный стресс в биопленке

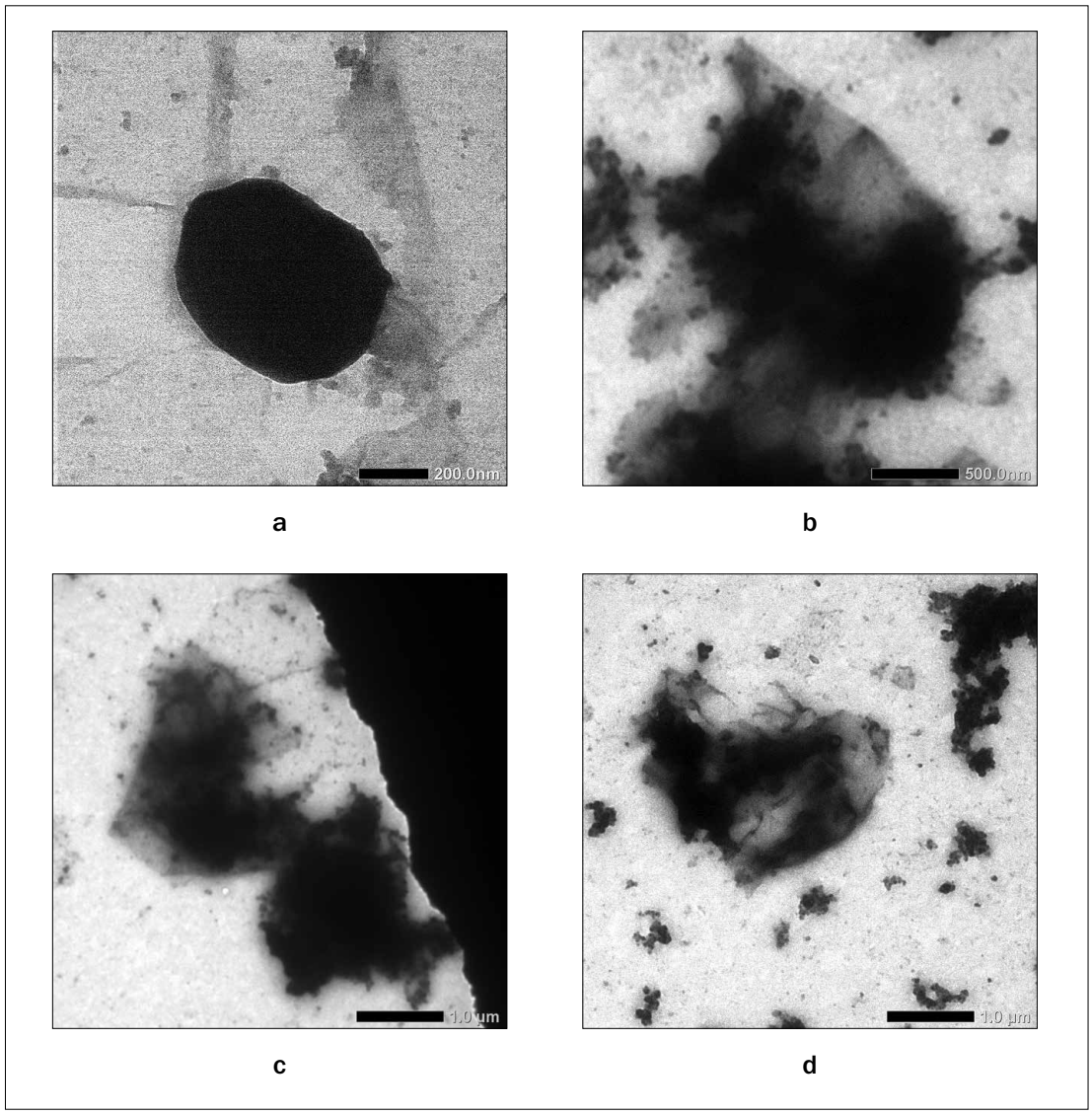

Fig. 7. Microscopic image of *Staphylococcus aureus* cells treated with laser, chlorophyll and ozone (×100000 magnification). (a) Normal cell (no treatment), (b) ROS reactions (including singlet oxygen, hydrogen peroxide, and superoxide anion radical) with cell membrane can cause extreme damage, (c) Damage to cell membranes causes the cytoplasm and cell organelles to become partly exposed and react directly with toxic molecules, (d) Cell organelles are directly exposed due to overall loss of the cell membrane
Рис. 7. Микроскопическое изображение клеток Staphylococcus aureus после воздействия лазеров, хлорофиллом и озоном (увеличение ×100000). (a) Здоровая клетка, (b) Воздействие активных форм кислорода (включая синглетный кислород, перекись водорода и супероксидный анионный радикал) на клеточную мембрану может привести к значительным повреждениям, (c) Повреждение клеточной мембраны частично приводит к непосредственному взаимодействию с токсичными молекулами, (d) Клеточные органеллы полностью открыты ввиду потери клеточной мембраны

glutathione (GSH) and albumin. Inactivation mechanisms occur when some nanoparticles diffuse into biofilm, bind to thiol (-SH) protein species because of high affinity and are affected by denaturation. The enzymes, carbohydrates, DNA and RNA could be affected depending on the ozone dose [14].

In comparison with ozone, the Ozone+Chlo group was not significantly different. There are no interactions between ozone and chlorophyll. Chlo+Ozone+Laser treatment gave higher biofilm reduction efficacy in contrast with ozone group or Ozone+Chlo group. It is fascinating to find out that particular treatment of chlorophyll and ozone concentration could enhance efficacy on PDI.

The mechanism of Chlo+Ozone+Laser group generates more toxic compounds to induce cellular damage. Onyango showed the reaction of that component could form toxic compound and generate ozone. Furthermore, the cytotoxic reactions occur continuously in this treatment. Therefore, we need to control the ozone dose for controlling the cytotoxic reaction [27]. Chlo absorbs the energy of laser, causing chlorophyll to be excited. Furthermore, photochemical type II reactions occur, in the

form of energy transfer from Chlo excited triplets to triplet oxygen. It is acknowledged that because of interaction between photosensitizer and light (Fig. 6), the superoxide anion ($^{\cdot}O_2^-$) and singlet oxygen ($^1O_2$) are formed. Each chlorophyll molecule produces approximately 10^3–10^5 singlet oxygen molecules before degrading due to photo bleaching or other processes [28]. The production of excess ROS can eliminate biofilms as protectors and cause oxidative stress of bacteria in biofilms.

Based on Fig. 7, the mechanism of cell destruction are due to generation of ROS including singlet oxygen, hydrogen peroxide, and superoxide anion radical. The normal cell of *Staphylococcus aureus* is a coccus and has slippery surface (Fig. 7a). After treatment, the normal cell has various damage starting from cell membrane. It causes the cytoplasm and cell organelles to react directly with toxic molecules. Grisham used the fluorescent method to detect H_2O_2 formation in the nucleus, mitochondria, endoplasmic reticulum and plasma membrane [29].

Conclusion

Combination of Chlo+Ozone+Laser treatment with high ozone exposure reduces biofilms by lesser amount. In this study, 3 treatment combinations at 20 s exposure to ozone showed increased biofilm reduction on average. Therefore, it is recommended to use a combination of laser, chlorophyll and lower ozone concentrations to increase the effectiveness of photodynamic inactivation.

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ORIGINAL ARTICLES

COMPARISON OF PHOTODYNAMIC THERAPY EFFICIENCY USING RADIATION SOURCES WITH DIFFERENT WAVELENGTHS IN THE TREATMENT OF SINUSITIS

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Abstract

Inflammatory diseases of the sinuses – one of the most common nosologies in the practice of otorhinolaryngologist; its pathogenesis is well studied, and treatment recommendations are detailed. Following them, however, cannot completely prevent chronic disease or recurrence. Antimicrobial photodynamic therapy is a promising method of treating sinusitis, which has proved its effectiveness, but has not yet been widely used. This work describes our experience in photodynamic therapy with chlorin e_6 of chronic sinusitis using a new laser diode-based irradiation source. For patients who had previous sinus surgery an adapter for penetration into the sinus through anastomosis was developed and tested. First group of the patients underwent photodynamic therapy (PDT) according to the conventional scheme, using a laser with a wavelength of 662 nm; while the second one underwent PDT using a 405 nm laser. With daily washing of the nasal sinus, the period of inflammation relief (evaluated by the absence of pathological discharge during washing) amounted to 3.8 full days on average in the first group of patients, compared to 5.4 days on average for the second group. We carried out the comparative analysis of the treatment results based on clinical assessment and radiological evaluation (CT) at the time of discharge from the hospital and 1 month later.

Keywords: chronic sinusitis, antibacterial photodynamic therapy, diode laser.

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СРАВНЕНИЕ ЭФФЕКТИВНОСТИ ФОТОДИНАМИЧЕСКОЙ ТЕРАПИИ ПРИ ИСПОЛЬЗОВАНИИ ИСТОЧНИКОВ ИЗЛУЧЕНИЯ С РАЗЛИЧНЫМИ ДЛИНАМИ ВОЛН В ЛЕЧЕНИИ СИНУСИТОВ

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Резюме

Воспалительные заболевания пазух носа – одна из самых распространенных нозологий с хорошо изученным патогенезом в практике оториноларинголога. Для ее лечения разработаны подробные рекомендации, следование которым, однако, не всегда позволяет полностью предотвратить переход заболевания в хроническую форму или возникновение рецидивов. Антимикробная фотодинамическая терапия (ФДТ) – перспективный метод лечения синуситов, уже доказавший свою эффективность, но еще не получивший широкого распространения в клинической практике. В работе описан опыт применения нового источника облучения на основе лазерных диодов, предназначенного для проведения ФДТ хронических синуситов с препаратами хлорина e_6. Для ранее прооперированных пациентов разработана и апробирована насадка для проникновения в пазуху через соустье. I-ой группе пациентов ФДТ проводили по общепринятой схеме с использованием лазера с длиной волны 662 нм, для облучения II-ой группы применяли источник облучения с длиной волны 405 нм. При ежедневном промывании носовой пазухи сроки купирования воспаления (оценивали по отсутствию патологического отделяемого при промывании) для пациентов I-ой группы составили в среднем 3,8 сут, а у пациентов II-ой группы – в среднем 5,4 сут. Проведен сравнительный анализ результатов лечения, которые оценивали клинически и рентгенологически на момент выписки и через 1 мес.

Ключевые слова: хронический синусит, антимикробная фотодинамическая терапия, диодный лазер.

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Introduction

Antimicrobial photodynamic therapy (APDT) is a successfully used method of treating inflammatory diseases based on the inactivation of pathogens caused by the interaction between a drug – a photosensitizer (PS) – and light with certain properties. The development of new types of photosensitizers and radiation sources contributes to the widespread use of APDT in various medical disciplines. For example, antimicrobial photodynamic therapy is used in otorhinolaryngology to treat acute and chronic sinusitis, chronic tonsillitis, chronic otitis, purulent diseases of the larynx [1, 2].

It has been proven that both bacteria and fungi, main pathogens causing the inflammation of mucous membranes of paranasal sinuses, form so-called biofilms [3, 4]. Their occurrence is a kind of defense mechanism that protects bacteria from the effects of the body's immune system and antibiotics. It has been shown that bacteria living in biofilms are more virulent than free-floating forms [5]. In recent decades, there were a lot of reports on the efficiency of the eradication of various microorganisms using APDT, including data on the efficient treatment of gram-negative and gram-positive antibiotic-resistant biofilms [6].

Chronic sinusitis is a polyetiological disease developing due to a combination of causes including congenital anomalies of the immune system, a disruption in the normal anatomy of the nasal cavity and environmental factors such as inflammatory and irritating agents. Common treatment algorithms are not always fully efficient, and the disease exacerbation rate does not decrease in a number of patients after the surgery for the restoration of the normal sinus drainage and aeration [7]. According to national and international standards, the exacerbation should be managed with systemic antibiotics therapy, which, if regularly repeated, cannot but increase the likelihood for antibiotic resistance [8]. In our opinion, PDT can play a preventive role here and serve as a method of treatment allowing to achieve a good effect in case of existing antibiotic resistance and having the potential to prevent and limit the increase in antibiotic resistance in a specific person and the whole population.

Photodynamic therapy of inflammatory chronic sinusitis is mainly intrasinus: the sinus of not operated patients is accessed by means of endonasal maxillary sinus dissection, while the sinus of operated patients is accessed through the dilated junction of the maxillary sinus.

A laser with a wavelength of 662 nm is usually used as an radiation source for PDT with chlorine e6 [9, 10]. However, there is an increasing amount of data on the use of other laser or LED sources but the clinical experience in application of such techniques is still limited [11]. The chlorine based PS has an absorption peak at around 405 nm, and the pilot study of LED irradiation with this wavelength gave a positive result [12], therefore we decided to conduct an in-depth study of its efficiency.

Materials and methods

22 patients (13 men and 9 women) with chronic purulent sinusitis in the exacerbation phase were treated in the ENT department of the Clinical Hospital No. 122 named after L. G. Sokolov of the FMBA of Russia from 2015 to 2017.

The eligibility criterion for patient enrollment in the study of PDT was the chronic maxillary sinusitis in the exacerbation phase confirmed by previous epicrises, findings of computed tomography of paranasal sinuses (the presence of pathological contents in maxillary sinuses) and endoscopic examination (the presence of edema, hyperemia of the nasal mucosa, purulent discharge in nasal meatuses). All patients had undergone surgical treatment for chronic sinusitis, after which the natural junction of the maxillary sinus in the middle nasal meatus or the artificial junction in the inferior nasal meatus remained dilated. Patients with chronic somatic diseases in the exacerbation or decompensation phase were excluded from the study. Exclusion criteria also included intake of antibiotics during the study for this sinusitis exacerbation episode or for any other reason and an advanced polipous process, which would fill the maxillary sinus.

Fotoditazin (VETA-GRAND LLC, Russia, registration licence No. LS 001246 of 18.05.2012) in the form of a concentrate for a solution for infusion of 5 mg/ml was used as a photosensitizer for PDT. On average, 7-9 ml of the PS solution were administered into each sinus, wherein the concentration of the solution for intrasinus administration corresponded to the average concentration for intravenous administration indicated in the package insert. The sinus was rinsed under topical anesthesia using a cannula, then it was blown out with air to release it completely from the liquid. After that, the prepared photosensitizer solution was administered. To prevent the solution leakage, the middle or common nasal meatus was plugged with a cotton tampon moistened with a fotoditazin solution in patients that had undergone infundibulotomy and maxillotomy, respectively. Topical anesthesia was not associated with rinsing itself but was used to ensure comfortable plugging.

ALCOM-MEDICA LLC (Russia) developed and manufactured a low-power unit with replaceable emitters, the "SHUTLE-COMBI IR+" complex based on diode lasers and LED light sources (Fig. 1). The complex allows simultaneous work with different types of low-energy sources. For this study we used laser emitters with working wavelengths of 662 nm and 405 nm. A special curved tip was attached to them to get into the artificial junction of the maxillary sinus (Fig. 2).

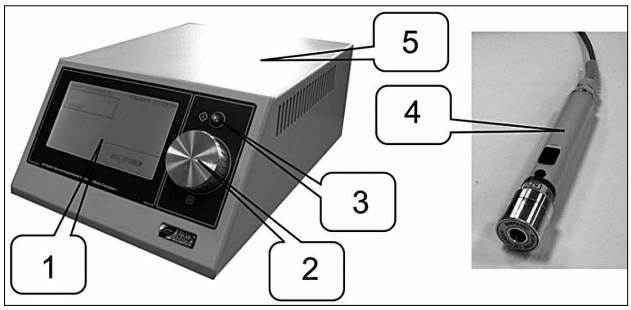

Рис. 1. Комплекс на основе диодных лазеров и светодиодных излучателей «ШАТЛ-Комби ИК+»:
1. графический дисплей с пультом управления
2. ручка регулировки
3. кнопка старт/стоп
4. излучатель
5. блок управления

Fig. 1. Complex based on diode lasers and LED emitters "SHUTLE-COMBI IR+":
1. graphic display with control panel
2. adjustment knob
3. start / stop button
4. emitter
5. control unit

Рис. 2. Насадка для верхнечелюстной пазухи
Fig. 2. Nozzle for maxillary sinus

The patients were divided into two groups. The sinuses of the first group of patients (9 people) were irradiated with a laser with a wavelength of 662 nm and an output power of 50 mW for 20 minutes. 13 patients of the second group were irradiated with a laser with a wavelength of 405 nm and an output power of 100 mW for 20 minutes.

The chosen PDT efficiency assessment criteria were the duration of management of inflammatory process exacerbation based on the presence and amount of purulent discharge from the sinuses, the length of patient's hospital stay and the findings of the X-ray inspection conducted at the end of treatment and repeated 1 month after release.

Results and discussion

Phototoxicity and allergic reactions were not observed in any patients during the study. The emitter was easy to use. The rigid tip allows applying this technique on previously operated patients with a wide junction of the maxillary sinus. Performance of this treatment using a fiber optic light cable would be difficult since the bending angle of the tip for sinus rinsing was large enough and led to the damage of the light cable. The results of inflammation management are shown in the table.

The length of patient's hospital stay corresponds to the daily duration of maxillary sinus rinsing.

The X-ray findings (according to helical computed tomography of the paranasal sinuses) at the end of the treatment and 1 month after it showed improvement in both groups of patients.

Thus, the results of PDT using a laser emitter with a wavelength of 662 nm are comparable with the literature data and confirm the efficiency of this method

Таблица
Сроки купирования воспалительного процесса (появление чистых промывных вод) после сеанса фотодинамической терапии
Table
Terms of the inflammatory process relief (the appearance of clean washing water) after a session of photodynamic therapy

Количество суток до появления чистых промывных вод Days before the appearance of clean washing water	Число пациентов с полностью купированным воспалительным процессом The number of patients with fully arrested inflammatory process			
	I группа (n=9) Group I (n=9)		II группа (n=13) Group II (n=13)	
	Абс Abs	%	Абс Abs	%
3	4	44,5		
4	4	44,5	2	15,5
5			5	38,4
6	1	11	5	38,4
7			1	7,7

in the treatment of sinusitis [10]. The use of an emitter with a wavelength of 405 nm during PDT showed good clinical outcomes: the inflammation was managed but over a longer period than at 662 nm. It should be noted that the work with this type of emitters shows better patient compliance since the majority of the population is familiar with a blue light lamp and its bactericidal activity, which makes it easier to explain the mechanism of action. The nature of discharge in all patients changed on the first day although they did not complete the course. We consider it as an opportunity to study the efficiency of irradiation with a source with a wavelength of 405 nm in bacterial cultures. It would also be interesting to study the efficiency of the light effect on biofilms at this wavelength. The depth of light penetration in tissues at a wavelength of 405 nm is small, so the use of violet light is reasonable when the abnormal focus is located at the surface. Since chlorine e6 based photosensitizers have an intense absorption peak in this spectral band, this significantly reduces the emitter power requirements making this type of irradiation advantageous for the use in the antimicrobial photodynamic therapy of sinusitis.

Conclusion

The described experience of clinical use shows that the laser irradiation at 405 nm is efficient as part of photodynamic therapy of chronic sinusitis and, along with laser irradiation at 662 nm, can be an alternative to the conventional management of sinusitis in the exacerbation phase. To obtain reliable statistical data, more patients should be enrolled, especially to study the efficiency of a 405 nm laser light source.

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EXPERIENCE OF USING PHOTODYNAMIC THERAPY IN THE TREATMENT OF ESOPHAGEAL CANCER

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Abstract

Photodynamic therapy (PDT) is a worthy alternative to surgical esophageal resection or endoscopic mucosal resection and dissection (EMR, ESD) in patients with superficial esophageal cancer with severe concomitant diseases as well as in patients with a common form of esophageal cancer with severe malignant dysphagia. Patients with superficial (7) and advanced (15) esophageal cancer received PDT as an independent method and as a supplement to complex treatment. Radachlorin was used as a photosensitizer at a dose of 0.6–0.8 mg/kg, administered intravenously 3 hours before irradiation. A PDT session was carried out using a laser with a wavelength of 662 nm. The light dose used was 150–300 J/cm^2. The use of PDT made it possible to achieve the full effect in 7 (100%) patients in the group of superficial (T1a-T1b) esophageal cancer where PDT was either the only method of treatment or in combination with radiation therapy. In the group of patients with stenotic cancer the use of PDT made it possible to achieve full recovery of food intake after recanalization for 20% of patients, and partial – for 66.7%. Thus, complete natural food intake was restored for 86.7% of patients which improved their quality of life. PDT is also a method of choice for cancer of the upper esophagus as esophageal stenting in this situation can cause unwanted subjective sensations.

Key words: photodynamic therapy, esophageal cancer, radachlorin,

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ОПЫТ ПРИМЕНЕНИЯ ФОТОДИНАМИЧЕСКОЙ ТЕРАПИИ В ЛЕЧЕНИИ РАКА ПИЩЕВОДА

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Резюме

Фотодинамическая терапия (ФДТ) является достойной альтернативой хирургической резекции пищевода и эндоскопической резекции или диссекции слизистой (EMR, ESD) у пациентов с поверхностным раком пищевода при наличии у них тяжелых сопутствующих заболеваний, а также у пациентов с распространенной формой рака пищевода при выраженной злокачественной дисфагии. Больные поверхностным (7 человек) и стенозирующим (15 человек) раком пищевода получали ФДТ в качестве самостоятельного лечения, а также в качестве дополнения к комплексной терапии. В качестве фотосенсибилизатора применяли препарат радахлорин в дозе 0,6–0,8 мг/кг, который внутривенно вводили пациентам за 3 ч до начала облучения. Сеанс ФДТ проводили с использованием лазера с длиной волны 662 нм. Световая доза составила 150-300 Дж/см^2. Применение ФДТ позволило достичь полного эффекта у 7 (100%) пациентов в группе с поверхностным (T1a-T1b) раком пищевода, где ФДТ применяли как единственный метод лечения или в сочетании с лучевой терапией. В группе пациентов со стенозирующим раком с помощью ФДТ удалось добиться полного восстановления питания после реканализации у 20% больных, частичного – у 66,7%. Таким образом, у 86,7% пациентов было восстановлено полноценное естественное питание, что значительно улучшило качество их жизни. ФДТ является методом выбора при лечении злокачественных

новообразований верхних отделов пищевода, так как стентирование пищевода в данной ситуации может вызвать нежелательные субъективные ощущения.

Ключевые слова: фотодинамическая терапия, рак пищевода, радахлорин.

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ORIGINAL ARTICLES

Introduction

Esophageal cancer (EC) is a difficult to treat disease that ranks the 8th in the structure of cancer morbidity in the world and the 6th in the structure of cancer mortality [1]. Despite the development of new diagnostic and treatment methods for esophageal cancer at the early stages in recent years, the frequency of detecting patients with EC at the third and fourth stages is still very high accounting for 67% [2]. An important indicator of the esophageal cancer malignancy is low 5-year survival rates, not exceeding 10–15% both in Russia and European countries [3]. In half of the cases, the main clinical symptom of esophageal cancer is dysphagia occurring when the lumen is narrowed by 50–70% [4] and limiting the possibilities of surgical, radiation, combination and comprehensive treatment [1, 2, 5]. In addition, there is a large group of patients (up to 25%) that cannot undergo curative surgery due to the presence of severe coexisting diseases and age-related changes [5]. Radiation therapy (RT) is an EC treatment method chosen for this patient population, however, in this case there is a high probability of the disease recurrence with the development of stenosis. Furthermore, the second course of RT is impossible after irradiation at doses corresponding to a curative treatment regimen [6]. Endoscopic treatment is increasingly used for these reasons. Endoscopic mucosa resection (EMR) and endoscopic submucosa dissection (ESD) are current standards of treatment for superficial esophageal cancer, especially when it is spreads within the mucosa [7]. However, when annular lesions spread to more than two thirds of the circumference of the esophagus, these techniques (EMR and ESD) are not recommended due to the high risk of persistent esophageal stricture formation after the therapy [7, 8]. Another urgent problem is the development of endoscopic methods for dysphagia resolution, allowing to restore oral alimentation and enhance the quality of life of patients with stenotic esophageal cancer [8, 9]. Currently, minimally invasive endoscopic technologies (dilatation, recanalization caused by exposure to electrolaser destruction, argon plasma coagulation or photodynamic therapy) and endoprosthesis replacement are the most promising methods [3, 5].

The possibilities of modern oncology have greatly expanded with the advent of photodynamic therapy (PDT). This is a unique two-component treatment method based on the use of photosensitizers (PS) activated by light [10, 11]. Photosensitizers accumulate in a malignant tumor and stay in it for a longer time than in healthy tissues. Under local laser irradiation with light of a certain wavelength (at the PS absorption peak), a photochemical reaction begins in the tumor forming singlet oxygen and oxygen free radicals, which have a toxic effect on malignant cells [4, 6, 7, 12, 13]. PDT is an alternative method of treating patients with stenotic esophageal cancer as well as patients with superficial malignant tumors of this localization that have contraindications to curative treatment.

Materials and methods

This article is based on a study, which was conducted in compliance with the provisions of the Declaration of Helsinki (1964, revised in 2013). The study was conducted with the approval of the Institutional Control Ethics Commission. The form of the informed consent to endoscopic manipulations, including PDT, was also approved at the meeting of the above. Patients were informed about the PDT method, its benefits and possible risks, treatment regimen and duration of hospitalization and follow-up examinations. All patients signed the informed consent before the treatment.

From 2015 to 2017, endoscopic photodynamic therapy was conducted in 22 patients with esophageal cancer. The patients were divided into two groups. The first one included 7 patients with superficial esophageal cancer (T1a,bN0M0) that refused surgical treatment or had a severe comorbidity or a residual tumor of esophagus after RT. PDT was used as a curative treatment method in this group. The second group included 15 patients with locally advanced esophageal cancer who received PDT as palliative care to enhance the quality of their life and increase their life expectancy. The condition for the use of PDT was the absence of deep carcinelcosis and an esophageal mediastinal or esophageal respiratory fistula. Men prevailed in both groups: the first group consisted of 5 men and 2 women, the second one included 15

men. The age of patients ranged from 47 to 76 years old and averaged 58 years old. In all cases, the squamous cell carcinoma was morphologically confirmed. Before the treatment, a four-degree scale of A.I. Savitsky was used to evaluate dysphagia:

the 1st degree of dysphagia is difficulty in swallowing solid food;

the 2nd degree of dysphagia is difficulty in swallowing semiliquid food;

the 3rd degree of dysphagia is difficulty in swallowing liquid food;

the 4th degree of dysphagia is difficulty in swallowing water, saliva.

In the first group of patients, no cases of dysphagia were detected; in the second one, there were the 2nd and the 3rd degrees of dysphagia. The lesion size varied: it ranged from 2 to 5 cm in patients of the first group and was up to 7 cm in patients of the second group. Localization of malignant esophageal tumors and tumor-induced stenosis was distributed as follows: in the middle third of the esophagus in 5 patients (71.4%) and in the lower third of the esophagus in 2 patients (28.6%) of the first group; in the upper third of the esophagus in 6 patients (40%), in the middle third of the esophagus in 8 patients (53.3%) and in the lower third of the esophagus in 1 patient (6.7%) of the second group. In the first group, PDT was used as an independent treatment method due to the presence of contraindications to curative surgical treatment or refusal of it in 3 (42.8%) patients or for the purpose of residual tumor destruction after RT in 4 (57.2%) patients. In the second group, PDT and subsequent endoprosthesis replacement (stenting) were conducted in 10 (66.7%) patients with the 3rd degree of dysphagia to recanalize tumor-induced stenosis. PDT was also conducted in 5 (33.3%) patients as an independent treatment method due to high location of tumors and inability to place a stent.

Before PDT, radachlorin (RADA-PHARMA LLC, Russia, registration licence No. LS-001868 of 16.12.2011) was administered to a patient via IV infusion at a dose of 0.6–0.8 mg/kg in a darkened room. A PDT session was conducted 3 hours after the PS administration using a laser with a wavelength of 662 nm (BIOSPEC LLC, Russia). A light dose was 150–300 J/cm^2. Irradiation was delivered to the site of exposure through a gastroscope channel. A quartz light guide was passed through an endoscope to a distal edge of the tumor, after which polypositional laser irradiation of the tumor was carried out. Quartz light guides with cylindrical diffusers and lengths of 1 to 5 m were used. The irradiation dose was selected individually depending on the tumor localization and size and the degree of esophagus narrowing. The number of irradiation positions ranged from 1 to 3. The total time of the polypositional irradiation of the tumor was from 10 to 40 minutes.

To prevent skin phototoxicity after endoscopic PDT, all patients were recommended to observe the light regime, involving limited exposure to the sun for 2 days and pain relief for 4 days if necessary. Follow-up esophagogastroduodenoscopy (EGD) was performed in the patients on the 4th day, then after 1 month and every 3 months after the PDT session. Computed tomography (CT) of the thoracic cavity was repeated every 3 months. In the presence of residual tumors, another PDT course was conducted 3-4 weeks after the previous one. Adverse events were evaluated during the first four days on the basis of patient complaints (pain behind the sternum, fever), visual examination and follow-up EGD performed on the 4th day and 1 month after the photodynamic therapy. The pain was assessed on a 10-point analogue scale and did not exceed 2–3 points on the 4th day. Intoxication syndrome was evaluated on the basis of patient complaints and data on fever. The PDT efficiency and the possibility of stenosis development were evaluated during esophagoscopy.

Results and discussion

The short-term treatment outcomes were evaluated on the 4th or 5th day after the first PDT course. During this period, demarcation of a necrotic zone and partial sloughing in a recanalization zone occurred, local inflammatory reaction remitted (Fig. 1).

In the first group, complete tumor regression was achieved in all patients as a result of PDT. With the full effect observed in 7 (100%) patients, unchanged mucosa was visible in 4 (57.2%) patients and scars were visible in 3 (42.8%) patients during the follow-up endoscopic examination. The absence of lesion growth signs was detected using a biopsy of the former tumor site and chromoendoscopy with a 1% Lugol's iodine solution.

The median follow-up of patients with superficial esophageal cancer was 28 months after the treatment. Progression in the form of distant metastases and local recurrence was diagnosed in only one patient who therefore underwent stent placement. The result was achieved in all 7 (100%) patients. In 2 (28.5%) patients with residual tumors after RT and achieving the full effect of PDT, cicatrical esophageal strictures of the 2nd degree were developed, which were successfully eliminated by means of endoscopic bougienage.

In the second group of patients, the effect was evaluated as complete and partial esophageal lumen restoration. The restoration of esophageal lumen was considered complete when it reached the diameter of 1 cm or more after recanalization (Fig. 2). Moreover, in the case of the complete esophageal lumen recanalization, an endoscope with a diameter of 9–12 mm smoothly reached the stomach. The complete esophageal lumen recanalization allowing patients to swallow almost any food was detected in 3 (20%) patients. The treatment effect in this

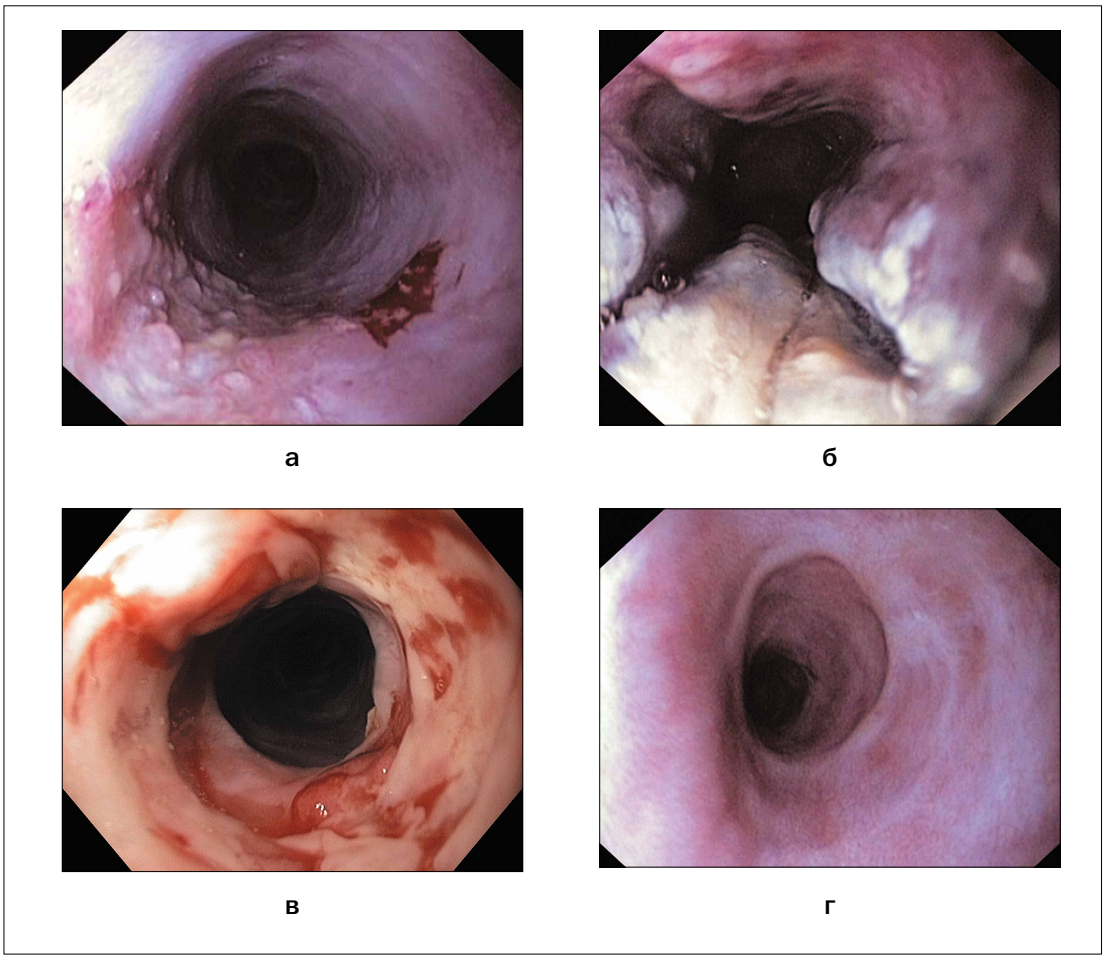

Рис. 1. Эндофотографии локализованной формы рака пищевода в различные сроки после ФДТ:
а – до проведения ФДТ;
б – 4-ые сутки после ФДТ;
в – через 1 мес после ФДТ;
г – через 30 мес после ФДТ

Fig. 1. Endophotographs of a localized form of esophageal cancer at various times after PDT:
а – before PDT;
б – 4th day after PDT;
в – 1 month after PDT;
г – 30 months after PDT

patient population was supported by multi-course PDT. During the study, one patient underwent a maximum of 6 PDT courses. Average survival duration of this patient population was 28.2 months.

The partial esophageal lumen restoration after PDT was observed in 10 (66.7%) patients. The restoration of esophageal lumen was considered partial if its diameter did not exceed 0.6–0.8 after recanalization. In addition, an endoscope could be only passed beyond the area of tumor-induced stenosis constrainedly or after an additional bougienage procedure. After partial recanalization, patients could swallow semiliquid or liquid food, which corresponded to the 2nd and the 3rd degrees of dysphagia. This group included patients with tumors located in the upper third of the esophagus and those who underwent PDT for recanalization before stent placement. Average survival duration of this patient population was 10.2 months.

The esophageal lumen recanalization procedure was not effective in 2 (13.3%) patients of the second group: after PDT and subsequent thermal destruction, the diameter of the esophagus remained the same, an endoscope could not be passed distal of stenosis, the patients' ability to swallow food did not change. This group included patients with tumor-induced stenosis of the upper third of the esophagus with extension to the larynx.

Conclusion

Thus, PDT is a valid alternative to surgical resection of the esophagus or endoscopic mucosa resection (EMR, ESD) in patients with superficial esophageal cancer having severe coexisting diseases as well as in patients with

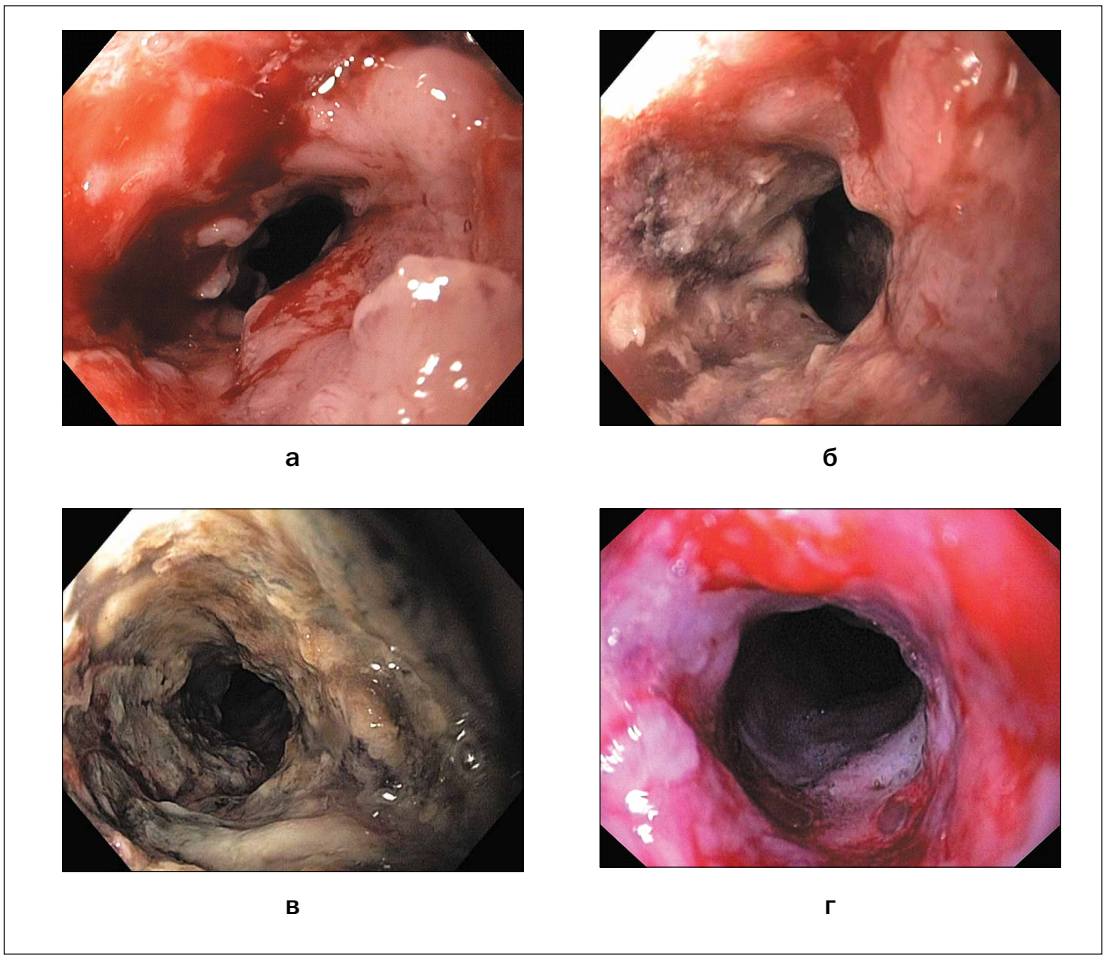

Рис. 2. Эндофотографии опухолевого стеноза пищевода в различные сроки после ФДТ:
а – до проведения ФДТ;
б – после ФДТ;
в – 4-ые сутки после ФДТ;
г – через 1 мес после ФДТ

Fig. 2. Endophotographs of tumor stenosis of the esophagus at various times after PDT:
а – before PDT;
б – after PDT;
в – 4th day after PDT;
г – 1 month after PDT

advanced esophageal cancer with severe malignant dysphagia. The use of PDT allowed for the full effect in 7 (100%) patients in the group with superficial (T1a-T1b) esophageal cancer where PDT was an exclusive treatment method or was combined with previously performed radiation therapy. In the group of patients with stenotic cancer, PDT was effective in 13 (86.7%) patients. In 3 (20%) of them, the esophageal lumen was completely restored, while in 10 (66.7%) of them, it was partially restored, which enhanced the quality of life and increased the survival duration of this patient population. PDT is also a treatment method chosen for cancer of the upper esophagus since esophageal stenting can cause undesirable subjective sensations.

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MODERN ASPECTS OF PHOTODYNAMIC THERAPY OF ACTINIC KERATOSES

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Abstract

Currently, photodynamic therapy (PDT) remains the most effective treatment for actinic keratosis (AK). With the increase in the incidence of AK, mainly due to the popularization of recreation in countries with increased insolation, there is an increasing interest in developing new methods of diagnostics and treatment and improving the existing ones. Studies that are aimed at determining the final efficacy of PDT, taking into account the resulting adverse reactions and long-term cosmetic results, are becoming increasingly popular. The nature of the light needed to excite a photosensitizer (PS) opens up new possibilities in the field of experimental studies that are aimed at reducing adverse reactions with similar efficacy of the applied therapy.

In the review article, we presented the results of our own and foreign studies on the diagnosis and treatment of AK for 2017–2019, namely: we determined the possibilities of using sources with natural and short-wave radiation at different depths of skin lesions; presented a classification of the growth of AK in the basal layer of the epidermis, which increases the possibility of predicting the outcomes of the disease; showed the prevailing efficiency of fluorescent diagnostics compared with traditional diagnostic methods; evaluated the advantages of PDT using natural light and artificial sources of radiation; described the possibility of using a combination of drugs to increase the effectiveness of PDT in difficult to treat areas and in AK foci with a high degree of damage to the basal layer of the epidermis.

Key words: actinic keratosis, keratinocytic intraepidermal neoplasia, squamous cell carcinoma in situ, photodynamic therapy, fluorescence diagnostics, photosensitizer, natural light, fotoditazin, aminolevulinic acid, cryosurgery.

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СОВРЕМЕННЫЕ АСПЕКТЫ ФОТОДИНАМИЧЕСКОЙ ТЕРАПИИ АКТИНИЧЕСКОГО КЕРАТОЗА

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Резюме

В настоящее время фотодинамическая терапия (ФДТ) является одним из наиболее эффективных методов лечения актинического кератоза (АК). По мере роста показателей заболеваемости АК, главным образом, из-за популяризации отдыха в странах с повышенной инсоляцией, возрастает интерес к разработке новых и усовершенствованию существующих методов диагностики и лечения. Всё более востребованными становятся исследования, которые направлены на определение конечной эффективности ФДТ с учётом возникших побочных реакций и отдалённых косметических результатов. Природа света, необходимого для возбуждения фотосенсибилизатора (ФС), открывает новые возможности в области экспериментальных исследований, которые направлены на

снижение частоты и степени выраженности побочных реакций при аналогичной эффективности применяемой терапии.
В обзорной статье приводятся результаты собственных и зарубежных исследований по диагностике и лечению АК за 2017–2019 гг.: определены возможности применения источников с естественным и коротковолновым излучением при различной глубине поражения кожи; представлена классификация роста очагов АК в базальном слое эпидермиса, увеличивающая возможность прогнозирования исходов заболевания; показана превалирующая значимость флуоресцентной диагностики (ФД) по сравнению с традиционными методами обследования; оценены преимущества применения ФДТ с использованием естественного света и искусственных источников облучения; описана возможность использования комбинации препаратов для повышения эффективности ФДТ на участках, плохо поддающихсялечению, и в очагах АК при высокой степени поражения базального слоя эпидермиса.

Ключевые слова: актинический кератоз, интраэпидермальная неоплазия кератиноцитов, плоскоклеточный рак кожи *in situ*, фотодинамическая терапия, флуоресцентная диагностика, фотосенсибилизатор, естественный свет, фотодитазин, аминолевулиновая кислота, криохирургия.

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Actinic keratosis (AK) is a precancerous disease of skin epidermal layer that arises from chronic exposure to ultraviolet radiation. The prevalence of AK is up to 8%, on average, in people over 40 years and tends to increase with age. It is known that in people with Fitzpatrick skin type I and type II, the risk of AK developing increases up to 40% [1-3]. Actinic keratosis is accompanied by atypical keratinocyte proliferation in the epidermis basal layer [4].

The relevance of the selected problem is determined by the risk of AK foci malignancy in squamous cell skin cancer. It is advisable to add the popularization of recreation in countries with high insolation, as well as such global problems of the modern population as obesity and alcohol consumption [5, 6] to existing AK risk factors. It is worth noting that in some cases, keratinocyte intraepidermal neoplasia is prone to spontaneous regression.

Obviously, a correctly chosen method of therapy and an assessment of the disease prognosis are the key to successful outcome of treatment. According to the researchers from the Ruhr University in Germany, the generally accepted histological classification (KINI – KINIII) does not determine the risks of malignancy of AK, so they worked to determine the depth of skin lesion that arises from keratinocyte intraepidermal neoplasia. According to the results of the completed study, it was proposed to identify the following types of AK foci growth:

– PRO I (crowding), characterized by the crowding of atypical keratinocytes in the epidermis basal layer;

– PRO II (budding) – budding of atypical keratinocytes in the upper papillary layer;

– PRO III (papillary sprouting) – atypical papillary keratinocytes sprouting in the upper dermis [8–9].

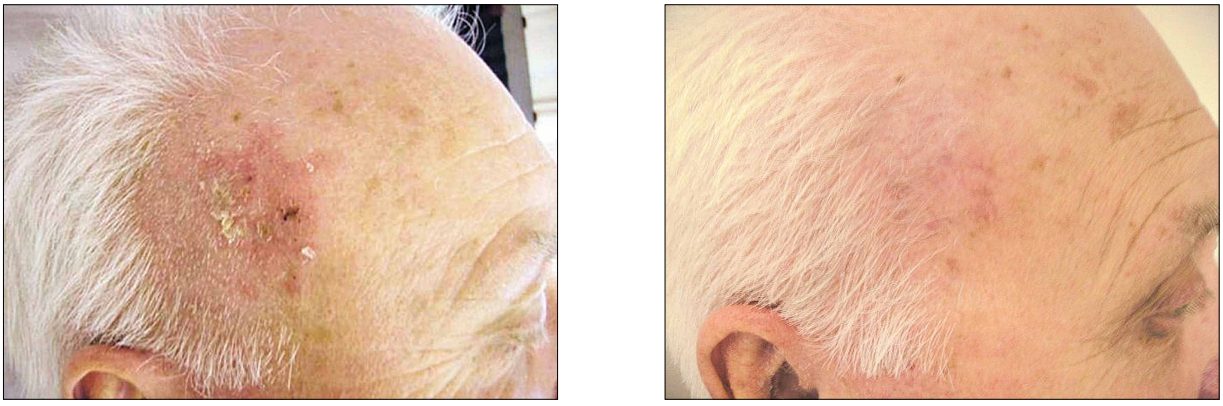
In the work of L. Schmitz and colleagues, it was proved that the risk of SCRC (squamous cell skin cancer) development depends on the AK foci growth pattern in epithelium basal layer [10]. The malignant potential of squamous carcinoma in situ involves early diagnosis and treatment to reduce disability and mortality. There are cases when it is difficult to carry out clinical and dermatoscopic assessment of skin lesions. The article describes 2 clinical cases of SCRC, uncertainly estimated as AK. The lack of the results of the therapy determined the conducting of supportive study of the areas by fluorescent diagnostics (FD) method, and the correct interpretation led to the accurate diagnosis verification. The findings of FD coincided with the results of a control histological study. The authors recommend using a non-invasive technique of confocal microscopy in the diagnosis of doubtful areas, as well as in the case of progression of neoplasia or in the absence of the response to the therapy [10].

G. Pellacani and co-authors from the University of Modena and Reggio Emilia arrived at similar conclusions after analyzing the results of AK treatment using confocal fluorescence microscopy after 5-fluorouracil injection. According to the researchers opinion, FD is a noninvasive alternative to the histological standard [12].

The objective of the authors' research, carried out in 2018, was to study the safety and response to local application of fotoditazin in AK photodynamic therapy (PDT). The study involved 80 patients with AK, represented by two experimental groups: treatment and control. The first group consisted of 40 patients with 151 AK foci (average age is 72 years). Figure 1 presents treatment response by the method of application of fotoditazin when exposed to PDT in given group of patients with AK.

The control group included 40 patients (average age is 65 years) with 64 AK foci treated with liquid nitrogen cryolysis, the results are shown in Fig. 2.

During PDT, the laser apparatus "LAMI" (OOO Novyye Khirurgicheskiye Tekhnologii, Russia) was used. 0.5% fotoditazin gel (VETA-GRAND, Russia) was used as a PS, having an absorption peak at 662 nm. Patients



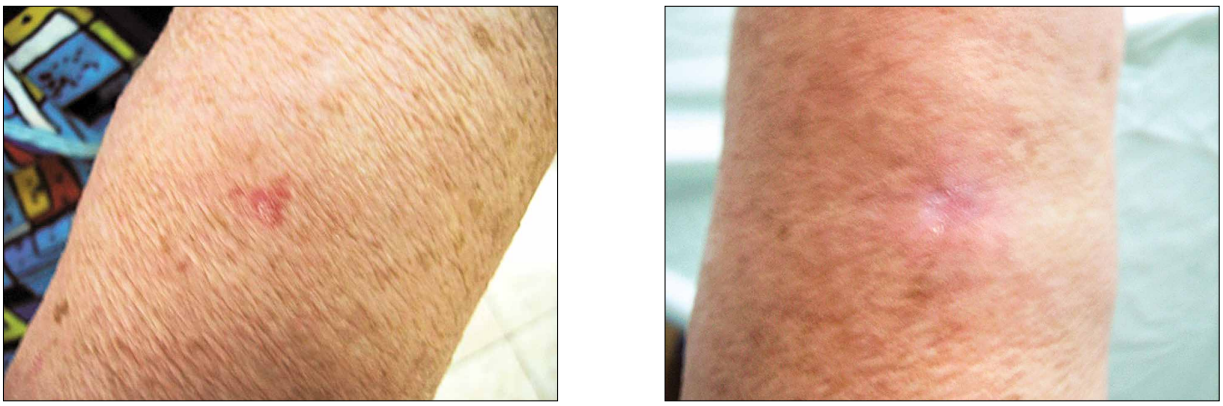
а б

Рис. 1. Очаг актинического кератоза:
а – до лечения;
б – через 3 мес после ФДТ
Fig. 1. Case of actynic keratoses:
а – before treatment;
б – 3 months after PDT

from treatment group received one PDT session after two-hour application of fotoditazin gel with the following irradiation parameters: light dose – 200 J/cm^2, power density – 0.14–0.48 W/cm^2. Patients from control group underwent AK foci cryolysis with liquid nitrogen using cryoprobe.

Two-year relapse-free survival in treatment group was 92.5%, and 85% in control group. Assessment of cosmetic results and adverse reactions of therapy was carried out after 24 months by the presence and severity of such reactions as: hyperemia, exudation, scarring, atrophy and indurations. For this, a visual analogue scale (VAS) was used, in which the value of 0 mm was rated as "very bad" and 100 mm – "very good". Cosmetic results were significantly higher after PDT ($p<0.05$) (Fig. 3), and the frequency and severity of adverse reactions from treatment were not statistically different.

In modern medicine, the qualitative and quantitative characteristics of factors aimed at reducing adverse reactions and improving the cosmetic results of therapeutic correction are of strategic importance. To this end, PDT AK studies are conducted abroad using various light sources. In most foreign studies, aminolevulinic acid (ALA) is used as a PS.



а б

Рис. 2. Очаг актинического кератоза:
а – до лечения;
б – через 3 мес после криодеструкции
Fig. 2. Case of actynic keratoses:
а – before treatment;
б – 3 months after cryosurgery

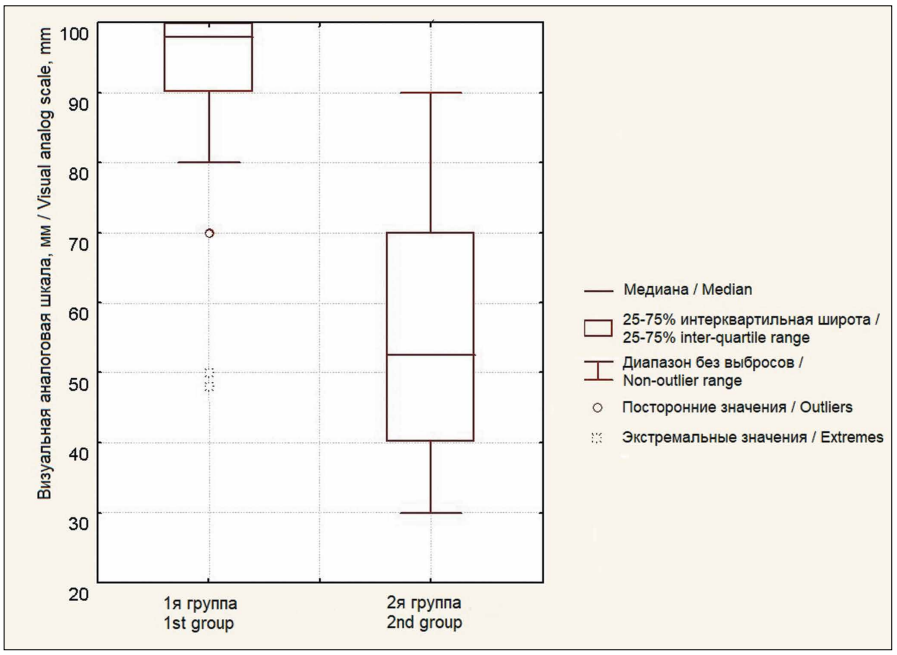


Рис. 3. Оценка косметических результатов в основной и контрольных группах через 24 мес после лечения
Fig. 3. Evaluation of cosmetic results in the main and control groups 24 months after the treatment

E. Kohl and colleagues carried out a research at the university hospital of Regensburg in Germany, which studied the response to cryolysis and PDT with 5-ALA when irradiated with natural light in the treatment of AK foci. Researchers believe that PDT with 5-ALA has a number of advantages over cryolysis, and the results of their work can be used in the treatment and prevention of AK [13].

A study on PDT with 5-ALA AK was published at Wroclaw Medical University, where PS excitation was performed with light of different wavelengths. The results of the study were evaluated after 9 months, the response to PDT with 5-ALA using red and green light was 92% and 87%, respectively. According to the authors, PDT with green light has the same response as AK foci irradiation with red light, but has lower frequency and pain severity [14].

The research work by P. Gholam and colleagues (Germany) presented the use of red and blue light sources. The authors determined response, tolerability, frequency of adverse reactions and cosmetic results of treatment with the most preferred sources of irradiation. PDT with 5-ALA AK was successful in 84% and 85% of cases, the intensity of pain assessed by VAS was 6.1 and 5.4 mm, respectively. According to the author's group, both sources of radiation demonstrate good results and can be used in PDT with 5-ALA AK [14].

Recently, more and more foreign researchers prefer the use of natural light in PDT with 5-ALA AK. For example, at the Aristotle University (Greece), therapeutic AK correction was performed by means of PDT with 5-ALA using natural and artificial irradiation sources. The results evaluated after 12 months after the treatment indicated no significant difference in response to the therapy (72% and 74%, respectively). At the same time, the researchers note that in the course of the treatment, patients prefer irradiation with natural light, due to the smaller number of adverse reactions recorded [16].

At the same time, a work to treat actinic cheilitis with natural light in PDT with 5-ALA was carried out in Rabin Medical Center (Israel). The study completed by scientists was highly valid, and the therapy was successful in 91% of cases [17].

The results of G.N. Galimberti work show high efficiency of PDT with 5-ALA with natural light in the medicinal use of 5-ALA and 5-fluorouracil gels. PS was used in concentrations of 16% and 5%. After 3 months, a regression of 9 affected foci occurred, which amounted up to 80% and 93%, respectively [18].

In the above works, the foci of keratinocyte intraepidermal neoplasia were located in the following areas: face, neck and shoulders. It is known that the treatment of affected areas of such localization has a good therapeutic prognosis, while AK foci located on notal side of the hands are difficult to treat. Scientists from the University of Copenhagen (Denmark) conducted a number of studies in which they used a combination of 5-ALA and 5-fluorouracil medications for the treatment of AK

foci located on hands. The results of exposure to only one 5-ALA showed significant response in 52% of cases, and the use of a combination of two drugs increased the response to the therapy to 63%. At the same time, the intensity of pain and the frequency of occurrence of skin erythema in different groups did not significantly differ [19].

Thus, in the face of increasing time spent by the population in the baking sun, combined with the risk factors for obesity and alcohol consumption, the problem of AK epidemiology is becoming more urgent. Currently, PDT is a global therapeutic vector in the choice of an innovative AK treatment method. To improve the qualitative and quantitative characteristics of the response to the therapy and cosmetic results, high compliance, as well as reduce adverse reactions, both new PS and new PDT techniques are being developed.

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SONODYNAMIC AND SONO-PHOTODYNAMIC THERAPY IN ONCOLOGY

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Abstract

In the present publication, authors have analyzed the results of using sonodynamic and sono-photodynamic therapy with photosensitizing agents of various classes (hematoporphyrin, 5-aminolevulinic acid, chlorin derivatives, etc.) in experimental oncology. In a number of *in vitro* and *in vivo* studies, the high antitumor efficacy of the above treatment methods has been proven. Ultrasonic treatment with a pulse frequency of 1–3 MHz and an intensity of 0.7 to 5 W/cm^2, independently and in combination with photo-irradiation of experimental tumors, can significantly improve the cytotoxic properties of photosensitizers. This became the basis for testing the methods in patients with malignant neoplasms of various localizations. Scientists from South-East Asia presented the preliminary results of the use of sonodynamic and sono-photodynamic therapy with photosensitizers in the treatment of malignant pathology of the mammary gland, stomach, esophagus, prostate, lung and brain. Analysis of the obtained data indicates the absence of serious adverse events and an increase in the antitumor efficacy of treatment, which included these treatment methods with chlorin-type photosensitizers.

Key words: sonodynamic therapy, sono-photodynamic therapy, photosensitizers, malignant tumors.

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СОНОДИНАМИЧЕСКАЯ И СОНО-ФОТОДИНАМИЧЕСКАЯ ТЕРАПИЯ В ОНКОЛОГИИ

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Резюме

В представляемой публикации авторами произведен анализ результатов применения сонодинамической и соно-фотодинамической терапии с фотосенсибилизирующими агентами различных классов (гематопорфирин, 5-аминолевулиновая кислота, производные хлорина и др.) в экспериментальных исследованиях и клинической онкологии. В ряде *in vitro* и *in vivo* исследований доказана высокая противоопухолевая эффективность указанных выше методов лечения. Ультразвуковое воздействие с частотой импульсов 1–3 МГц и интенсивностью от 0,7 до 5 Вт/см^2 в отдельности и в комбинации с фотооблучением экспериментальных опухолей, позволяет существенно повысить эффективность лечения. Это стало основой для апробации методов у пациентов со злокачественными новообразованиями различных локализаций. Учеными из стран Юго-Восточной Азии представлены предварительные результаты применения сонодинамической и соно-фотодинамической терапии с фотосенсибилизаторами в лечении злокачественной патологии молочной железы, желудка, пищевода, предстательной железы, легкого и головного мозга. Анализ полученных данных свидетельствует об отсутствии серьезных нежелательных явлений и повышении противоопухолевой эффективности лечения, в которое были включены данные методы лечения с фотосенсибилизаторами хлоринового ряда.

Ключевые слова: сонодинамическая терапия, соно-фотодинамическая терапия, фотосенсибилизаторы, злокачественные новообразования.

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Introduction

Currently, the possibility to use ultrasonic radiation (US) as an antitumor agent is studied extensively. The study of biological effects of ultrasonics with various frequencies, intensities and durations of action showed that US has a corresponding activity [1, 2].

According to some authors, US with a pulse rate of 0.5–3 MHz and an intensity of 0.5–5 W/cm^2 can increase cytotoxicity of various chemotherapeutic agents associated with an increase in permeability of the cell membranes and action of effects such as cavitation, hyperthermia and sono-induced free-radical oxidation of the tumor cell biological structures [3–5]. The new specialty has been called "sonodynamic therapy" (SDT), and such agents are commonly called sonosensitizers (SS). Radiosensitizers (dimexide, metronidazole) and a number of chemotherapeutic agents (bleomycin, adriamycin, cisplatin, etoposide, 5-fluorouracil, etc.) belong to the SS class in the first place [6, 7].

However, in the early 1990s, a research group from Tokyo guided by T. Yumita [8] published the early results confirming the high efficiency of SDT with a photosensitizer (PS) hematoporphyrin.

In recent years, results of in vitro and in vivo studies have been published showing great antitumor effects of the proposed method in the treatment of various specific forms of malignant tumors (breast cancer, lung cancer, liver cancer, colorectal cancer, pancreatic cancer, soft tissue sarcoma, skin melanoma, osteosarcoma, ascitic forms of ovarian neoplasms, leukemias, gliomas [4, 9]). The key findings of these studies are listed in Tables 1 and 2.

In a number of publications, authors provided data on synergistic enhancement of the cytotoxicity of photosensitizers on combined exposure of several physical factors (ultrasonic and laser radiation) to a sensitized tumor cell [1, 4, 32–34]. This specialty was called sono-photodynamic therapy (SPDT). The results of main studies using SPDT with sonodynamic exposure are shown in Table 3.

The main mechanisms of SPDT

The antitumor sono-photodynamic effect is based on reactions that develop:

1. during photodymamic therapy: – direct cytotoxic effects due to free-radical oxidation of biological structures (Fig. 1) [43, 44];

– disturbance in the blood supply to the tumor tissue due to damage to the endothelium of blood vessels feeding the tumor (Fig. 1) [43-45];

– activation of components of the immune system (Fig. 1) [43-45].

2. during sonodynamic therapy:

– physical processes (Fig. 2) [1, 2, 46];

– physicochemical processes (Fig. 2) [1, 2, 46];

– biological reactions (Fig. 2) [1, 2, 46].

The results of all the above reactions proceeding in a tumor cell on combined exposure of photosensitizing agents, ultrasonic and laser radiation are apoptosis, necrosis and autophagy [1, 2, 43–46].

Apoptosis develops due to the action of sonodynamic and photodynamic effects at a low radiation intensity, while necrosis develops when high-intensity radiation is used. In the former case, the triggering mechanism is disruption of lysosomal and mitochondrial membranes leading to the rapid release of mitochondrial cytochrome C into cytosol followed by the apoptosome and procaspase-3 activation [1,2, 43–46]. In the latter case (specific to photodynamic effects), the triggering mechanism is damage to components of the tumor microvasculature with the development of vascular stasis, thrombosis and congestion. According to most authors, the key factor triggering the necrosis process is the formation of an increased concentration of Ca2+ ions in cytoplasm due to disruption of mitochondrial membranes and endoplasmic reticulum. The above mentioned ions activate cysteine proteinases – calpains – leading to the destruction of lysosomes and the release of lysosomal enzymes (cathepsins) followed by the start of calpain-cathepsin necrosis pathway driving tumor cell death [43, 44].

Clinical testing of SPDT in patients with malignant tumors

A few authoring teams make the first attempts to use SDT and SPDT in a clinical setting. Preliminary results have been obtained showing the efficiency and safety of the proposed method in the treatment of metastatic breast cancer, head and neck tumors, colorectal cancer, lung tumors, esophageal cancer, prostate tumors [33, 47-49].

T. Inui et al. reported on the successful follow-up of a patient with terminal breast cancer with skin invasion after previous surgical treatment (October 2011) treated with Gc protein-derived macrophage-activating factor (GcMAF; intramuscularly; 0.5 ml 2 times a week, 21 administrations), hormonotherapy with the aromatase inhibitor Exemestane (Aromasin, 25 mg/day, orally) and 19 sessions of SDT with chlorine e6 (25 mg/kg, intravenously) and 5-aminolevulinic acid (10 mg/kg, orally) photosensitizers.

In January 2013, the progression of the disease was detected in the patient on the basis of suction biopsy data with the development of corresponding clinical picture (cough, pain, swelling right arm). The results of PET/CT (June 2013) showed the presence of a metastatic tumor of soft tissues in the axillary region, permeation along the spinal cord, an intrapleural nodular tumor component and metastatic pleurisy on the right side. After the conservative treatment, the regimen of which is indicated above, the PET/CT examination data (September 2013) showed a significant decrease in the size of tumors in axillary and intrapleural regions. Signs of metastatic pleurisy were not detected. No serious adverse events were observed [48].

X. Wang et al. reported on the outcomes of treatment of 3 patients with metastatic breast cancer treated using

Таблица 1
Применение сонодинамической терапии в эксперименте *in vitro*
Table 1
Application of sonodynamic therapy in an *in vitro* experiment

Авторы Authors	Штамм опухоли Tumor strain	ФС, доза (мг/кг) PS, dose (mg/kg)	Параметры ультразвука Ultrasound parameters	Эффективность Efficacy
Xiong W. et al., 2015 [10]	Саркома 180 Sarcoma 180	Синопорфирин натрия, 0,05 мкг/мл Synoporphyrin sodium, 0.05 mkg/ml	1,1 МГц 2 Вт 0,5; 1 и 1,5 мин 1,1 MHz 2 W 0.5; 1 and 1.5 minutes	Количество жизнеспособных клеток: ФС + УЗ (0,5 мин) – 63,54%; ФС + УЗ (1 мин) – 48,79%; ФС + УЗ (1,5 мин) – 19,55% (p<0,05). Количествоапоптотических клеток: контроль – 4,1%; ФС + УЗ (0,5 мин) – 44,2%; ФС + УЗ (1 мин) – 49,15%; ФС + УЗ (1,5 мин) – 79,5% (p<0,05). Amount of viable cells: PS + US (0.5 minute) – 63.54%; PS + US (1 minute) – 48.79%; PS + US (1.5 minute) – 19.55% (p<0.05) Amount of apoptotic cells: PS + US (0.5 minute) – 44.2%; PS + US (1 minute) – 49.15%; PS + US (1.5 minute) – 79.5% (p<0.05)
Sun H.et al., 2015 [11]	Адено-карцинома эндометрия Ishikawa, HEC-1α Endometrial adeno-carcinoma, Ishikawa, HEC-1α	Гемато-порфирин, 15 и 50 мкг/мл Hematoporphyrin, 15 and 50 mkg/ml	1 МГц, 1 Вт/см^2; 1 мин – Ishikawa; 1 МГц, 2 Вт/см^2, 4 мин – HEC-1α 1 MHz, 1 W/cm^2, 1 minute – Ishikawa; 1 MHz, 2 W/cm^2; 4 minutes – HEC-1α	Количество жизнеспособных клеток: Ishikawa: контроль – 100%; ФС– 85%; УЗ – 45%; ФС + УЗ – 25% (p<0,01). HEC-1α: контроль – 100%; ФС– 50%; УЗ – 80%; ФС + УЗ – 15% (p<0,01). Количество апоптотических клеток: Ishikawa: контроль – 0%; ФС – 10%; УЗ – 90%; ФС + УЗ – 95% (p<0,05). HEC-1α: контроль – 10%; ФС– 45%; УЗ – 20%; ФС + УЗ – 70% (p<0,01). Amount of viable cells: Ishikawa: control – 100%, PS – 85%, US – 45%, PS +US – 25% (p<0.01) HEC-1α: control – 100%, PS – 50%, US – 80%, PS + US – 15% (p<0,01). Amount of apoptotic cells: Ishikawa: control – 0%, PS – 10%, US – 90%, PS +US – 95% (p<0.05) HEC-1α: control – 10%, PS – 45%, US – 20%, PS + US – 70% (p<0,01).

Li Y.N. et al., 2015 [12]	Остеосаркома UMR106 Osteosarcoma UMR106	5-аминолевулиновая кислота(5-АЛК), 2 мкг/мл 5-aminolevulinic acid (5-ALA), 2 mkg/ml	1 МГц, 2 Вт/см^2, 7 мин 1 MHz, 2 W/cm^2, 7 minutes	Количество апоптотических клеток в группе ФС + УЗ – 27,2±3,4% (p<0,05); Активные формы O_2 – 32,6±2,2% (p<0,05) по сравнению с контролем, УЗ, ФС. Amount of apoptotic cells: in PS + US group – 27.2±3.4% (p<0.05) Reactive oxygen species – 32.6±2.2% (p<0.05) in comparison with control, US and PS.
Hu Z. et al., 2015 [13]	Меланома Melanoma	5-АЛК, 2 мкг/мл 5-ALA, 2 mkg/ml	1 МГц, 1,5 Вт/см^2 1 MHz, 1.5 W/cm^2	В группе ФС + УЗ: количество апоптотических клеток, % генерации активных форм O_2достоверно выше (p<0,05). Отмечена сверхэкспрессия гена miR-34a (в 16 раз выше, чем в группах сравнения). Снижение уровня антиапоптотических факторов (BCL2, CCND1, CDK6, SIRT1). In PS + US group: amount of apoptotic cells, % of reactive oxygen species generation is significantly higher (p<0.05). Marked overexpression of the miR-34a gene (16 times higher than in comparison groups). Reduced anti-apoptotic factors (BCL2, CCND1, CDK6, SIRT1).
Liu X. et al., 2015 [14]	Остеосаркома MG-63 Osteosarcoma MG-63	Гемато-порфирин, 20 мкг/мл Hemato-porphyrin, 20 mkg/ml	1 Вт/см^2, 0,5 мин 1 W/cm^2, 0.5 minutes	В группе ФС + УЗ отмечено достоверное увеличение интенсивности апоптоза (прокаспаза-3, каспаза 3 и 9). In the PS + US group, a significant increase in the intensity of apoptosis was noted (pro-caspase-3, caspase 3 and 9).
Wang X. et al., 2015 [15]	Гепато-целлюлярная карциномаHepG2 Hepatocellular carcinoma HepG2	Гипокреллин В, 2,5 мкг/мл Hypocrellin B, 2.5 mkg/ml	0,46 Вт/см^2, 8 с 0.46 W/cm^2 8 seconds	В группе ФС + УЗ выявлено более интенсивное повреждение митохондрий, лизосом, комплекса Гольджи, эндоплазматического ретикулума. In the PS + US group, more intense damage to mitochondria, lysosomes, the Golgi complex, and the endoplasmic reticulum was detected.
Xiang J.et al., 2014 [16]	Карцинома яичников человека НО-8910 Human ovarian carcinoma HO-8910	Метиленовый синий, 100 мкг/мл Methylene blue, 100 mkg/ml	1,7 МГц, 0,46 Вт/см^2, 5 с 1.7 MHz, 0.46 W/cm^2, 5 seconds	Количество колоний НО-8910 в группах: ФС + УЗ – 4 (p<0,05); ФС – 30; УЗ – 25. The number of HO-8910 colonies in groups: PS + US – 4, PS – 30, US – 25 (p<0.05).

Dai S. et al.2014 [17]	Глиома С6 Glioma C6	Гемато-порфирин, 20 мкг/мл Hemato-porphyrin, 20 mkg/ml	0,6; 0,8 и 1 МГц, 1 Вт/см^2, 1 мин 0.6, 0.8 and 1 MHz, 1 W/cm^2. 1 minute	Количество жизнеспособных клеток: при УЗ (0,6 МГц) – 43,2±3,2% при УЗ (0,8 МГц) – 57,1±3,7% при УЗ 1 МГц – 60,2±2,6%. Количество апоптотических клеток: контроль – 4,2±0,5% УЗ – 16±0,8%; ФС+УЗ – 49,4±2,6% (p<0,05). Amount of viable cells: US (0.6 MHz) – 43.2±3.2% US (0.8 MHz) – 57.1±3.7% US (1 MHz) – 60.2±2.6% Amount of apoptotic cells: control – 4.2±0.5% US – 16±0.8%; PS + US – 49.4±2.6% (p<0.05)
Li Y.J. et al. 2014 [18]	Рак поджелудочной железы Capan-1 Pancreas carcinoma Capan-1	5-АЛК, 5 мкг/мл 5-ALA, 5 mkg/ml	1 МГц, 2 Вт/см^2, 5 мин 1 MHz, 2 W/cm^2, 5 minutes	Количество жизнеспособных клеток: контроль – 100%; УЗ – 85±5,2%; ФС + УЗ – 59,2±7,9% (p<0,05). Количество апоптотических клеток: контроль – 9,1±1,2%; ФС – 9,5±1,2% (p=0,078); УЗ – 13,1±1,5%; ФС + УЗ – 34,6±5,6% (p<0,001). Amount of viable cells: control – 100%, US – 85±5.2%, PS + US – 59.2±7.9% (p<0.05) Amount of apoptotic cells: control – 9.1±1.2%, PS – 9.5±1.2% (p=0.078), US – 13.1±1.5%, PS + US – 34.6±5.6% (p<0.001).
Su X. et al. 2014 [19]	Миелогенная лейкемия человека К562 Human myelogenous leukemia K562	Протопорфирин, 5 мкг/мл Protoporphyrin, 5 mkg/ml	1,1 МГц, 1 Вт/см^2, 1 мин 1.1 MHz, 1 W/cm^2, 1 minute	Количество жизнеспособных клеток: контроль – 100%; ФС – 91,1% (p>0,05); УЗ – 85,9% (p>0,05); ФС + УЗ – 39% (p<0,01). Активные формы O_2: ФС – 10,13% (p>0,05); УЗ – 5,47% (p>0,05); ФС + УЗ – 23,87% (p<0,01). Amount of viable cells: control – 100%, PS – 91.1% (p>0.05), US – 85.9% (p>0.05), PS + US – 39% (p<0.01) Reactive oxygen species: PS – 10.13% (p>0.05), US – 5.47% (p>0.05), PS + US – 23.87% (p<0.01).

Chen B. et al. 2013 [20]	Аденокарцинома легких человека SPCA-1 Human lung adenocarcinoma SPCA-1	Хлорин e_6, 0,2 мг/мл Chlorin e_6, 0,2 mg/ml	1 МГц, 1 Вт/см^2, 1 мин 1 MHz, 1 W/cm^2, 1 minute	Количество некротических клеток: ФС – 3,73±0,34%; УЗ – 17,62±1,1%; ФС + УЗ – 74,23±1,02% (p<0,05). Amount of necrotic cells: PS – 3.73±0.34%, US – 17.62±1.1%, PS + US – 74.23±1.02% (p<0.05).
Su X. et al. 2013 [21]	Миелоидная лейкемия человека U937 Myelogenous leukemia human U937	Гемато-порфирин, 5 мкг/мл Hemato-porphyrin, 5 mkg/ml	1,1 МГц, 1 Вт/см^2, 1 мин 1.1 MHz, 1 W/cm^2, 1 minute	Количество жизнеспособных клеток контроль – 100%; ФС – 95,1%; УЗ – 82,99%, ФС+УЗ – 45,4% (p<0,05). Количество апоптотических клеток: ФС – 4%; УЗ – 15,8%; ФС + УЗ – 35,6% (p<0,05). Amount of viable cells: control – 100%, PS – 95.1%, US – 82.99%, PS+US – 45,4% (p<0.05). Amount apoptotic cells: PS – 4%, US – 15.8%, PS + US – 35.6% (p<0.05).

Таблица 2
Применение сонодинамической терапии в эксперименте *in vivo*

Table 2
Application of sonodynamic therapy in *in vivo* experiments

Авторы Authors	Штамм опухоли, животные Tumor strain,animals	ФС,доза (мг/кг) PS,dose (mg/kg)	Параметры ультразвука Ultrasound parameters	Эффективность Efficacy
Xiong W. et al., 2015 [10]	Саркома 180 мыши BALB/c Sarcoma 180 Mice BALB/c	Синопорфирин натрия, 2 мг/кг Sinoporphyrin sodium, 2 mg/kg	1,9 МГц, 4 Вт/см^2 1.9 MHz, 4 W/cm^2	Коэффициент торможения роста опухоли: ФС – 19,71%;УЗ – 32,56%; ФС + УЗ – 89,92% (p<0,01) The rate of tumor growth inhibition: PS – 19.71%, US – 32.56%, PS + US – 89.92% (p<0.01)
Foglietta F. et al., 2015 [22]	Аденокарцинома молочной железы Mat B III Крысы Wistar Mammary adenocarcinoma Mat B III Wistar rats	5-АЛК, 375 мг/кг 5-ALA, 375 mg/kg	Импульсный режим: 0,88 мДж/мм^2, 500 импульсов, 4 импульса/с Pulse mode 0.88 mJ/mm^2, 500 pulses, 4 pulses per second	7 Т МРТ через 72 ч после лечения: объем опухоли (см^3): контроль – 2,08±0,2; УЗ – 1,64±0,28; ФС – 1,56±0,74; ФС + УЗ – 0,79±0,39 (p<0,05) 7 T MRI 72 hours after treatment:tumor volume (cm^3): control – 2.08±0.2, US – 1.64±0.28, PS – 1.56±0.74, PS + US – 0.79±0.39 cm^3 (p<0.05)

Li Y. et al., 2015 [23]	Остеосаркома UMR106 Крысы Wistar Osteosarcoma UMR106 Wistar rats	5-АЛК, 250 мг/кг 5-ALA, 250 mg/kg	1 МГц, 2,5 Вт/см^2, 7 мин 1 MHz, 2.5 W/cm^2, 7 minutes	Объем опухолей на 10-е сутки после лечения – в группе ФС + УЗ – 400 (мм^3), что достоверно больше, чем в контроле ($p<0,01$), ФС и УЗ ($p<0,05$) The volume of tumors on 10th day after treatment: in the PS + US group – 400 mm^3, which was significantly higher than in the control ($p<0.01$), PS and US groups ($p<0.05$)
Alomol-hoda M. et al., 2015 [24]	Спонтанная адено-карцинома молочной железы Мыши BALB/c Spontaneous mammary adeno-carcinoma Mice BALB/c	Гемато-порфирин, 10 мг/кг Hemato-porphyrin, 10 mg/kg	0,15 МГц, 0,2 Вт/см^2 + 1 МГц, 2 Вт/см^2, 30 мин Фракции УЗ на 1,6,12,18 сут 0.15 MHz, 0.2 W/cm^2 + 1 MHz, 2 W/cm^2, 30 minutes Sonication on the 1st, 6th, 12th and 18th day	Коэффициент торможения роста опухоли в группе ФС + УЗ (4 фракции) был на 50% выше, чем в других группах ($p<0,05$) The rate of tumor growth inhibition in the PS + US group (4 fractions) was 50% higher, than in other groups ($p<0.05$)
Hu Z. et al., 2015 [25]	Меланома Мыши BALB/c Melanoma BALB/c mice	5-АЛК, 250 мг/кг 5-ALA, 250 mg/kg	1 МГц, 2 Вт/см^2, 5 мин 1 MHz, 2 W/cm^2, 5 minutes	Коэффициент торможения роста опухоли был достоверно выше в группе ФС + УЗ ($p<0,05$) The rate of tumor growth inhibition was reliably higher in the PS + US group ($p<0.05$)
Song D. et al., 2014 [26]	Глиома с6 Крысы Wistar Glioma c6 Wistar rats	Гемато-Порфирин, 10 мг/кг Hemato-porphyrin, 10 mg/kg	1 МГц, 0,5 Вт/см^2, 2 мин 1 MHz, 0.5 W/cm^2, 2 minutes	Объем опухоли на 14-е сутки после лечения: ФС – 125 мм^3; УЗ – 100 мм^3; ФС + УЗ – 60 мм^3 ($p<0,05$) Tumor volume on the 14th day after treatment: PS – 125 mm^3, US – 100 mm^3, PS + US – 60 mm^3 ($p<0.05$).
Li C. et al., 2014 [27]	Саркома S180 Мыши BALB/c Sarcoma S180 BALB/c mice	DVDMS, 1,2 и 4 мг/кг DVDMS, 1.2 and 4 mg/kg	1,9 МГц, 3 мин 1.9 MHz, 3 minutes	Коэффициент торможения роста опухоли на 14-е сутки: ФС – 22,81%; УЗ – 25,67%; ФС + УЗ – 56,27% ($p<0,05$). The rate of tumor growth inhibition on the 14th day: PS – 22.81%, US – 25.67%, PS + US – 56.27% ($p<0.05$).

Wang S. et al. 2014 [28]	Murine melanoma B16F10 Мыши BALB/c и nude Murine melanoma B16F10 BALB/c and Nude mice	5-АЛК, 250 мг/кг 5-ALA, 250 mg/kg	1,1 МГц, 2 Вт/см^2, 5 мин 1.1 MHz, 2 W/cm^2, 5 minutes	Коэффициент торможения роста опухоли на 11-е сутки: УЗ (BALB/c) – 35,95%; УЗ (nude) –31,36% ФС + УЗ (BALB/c) – 60,94%; ФС + УЗ (nude) – 59,89% (p<0,05). The rate of tumor growth inhibition on 11th day: US (BALB/c) – 35.95%, US (nude) – 31.36%, PS + US (BALB/c) – 60.94%, PS + US (nude) – 59.89% (p<0.05).
Gao G. et al., 2013 [29]	SAS Мыши BALB/c SAS Mice BALB/c	5-АЛК, 250 мг/кг 5-ALA, 250 mg/kg	1,1 МГц, 2 Вт/см^2, 5 мин 1.1 MHz, 2 W/cm^2, 5 minutes	Коэффициент торможения роста опухоли на 14-е сутки: УЗ – 22,38%; ФС + УЗ – 43,77% (p<0,05). The rate of tumor growth inhibition on 14h day: US – 22.38%, PS + US – 43.77% (p<0.05).
Chen B. et al. 2013 [30]	Адено-карцинома легких человека SPCA-1, Мыши Kunming Human lung adenocarcinoma SPCA-1 Kunming mice	Хлорин e_6, 10; 20; 40 мг/кг Chlorin e_6, 10; 20 and 40 mg/kg	0,4; 0,8; 1,6 МГц, 1,6 Вт/см^2 0.4, 0.8 and 1.6 MHz, 1.6 W/cm^2	Максимальный противоопухолевый эффект: ФС 40 мг/кг + УЗ 1,6 Вт/см^2 Maximum antitumor efficacy: PS 40 mg/kg + US 1.6 MHz
Yamaguchi F. et al., 2013 [31]	Глиобластома человека U87MG Мыши BALB/c Human glioblastoma U87MG BALB/c mice	5-АЛК, 100 мг/кг 5-ALA, 100 mg/kg	25 кГц, 4 Вт/см^2, 4 мин 25 KHz, 4 W/cm^2, 4 minutes	Средний объем опухоли на21-е сутки: контроль – 6,89±1,19 мм^3; ФС – 4,85±1,59 мм^3; УЗ – 5,08±2,77 мм^3; ФС + УЗ – 0,08±0,08 мм^3 (p<0,05). Mean tumor volume on 21st day: control – 6.89±1.19 mm^3, PS – 4.85±1.59 mm^3, US – 5.08±2.77 mm^3, PS + US – 0.08±0.08 mm^3 (p<0.05).

Таблица 3
Применение соно-фотодинамической терапии в экспериментах *in vitro* и *in vivo*

Table 3
Application of sono-photodynamic therapy in *in vitro* and *in vivo* experiments

Авторы Authors	Штамм опухоли, животные Tumor strain, animals	ФС, доза (мг/кг) PS, dose (mg/kg)	Параметры: ультразвук/ фотооблучение (ФО) Parameters: ultrasound/ photo- irradiation (PI)	Эффективность Efficacy
	In vitro			
Bakh-shizadeh M., et al., 2017 [35]	Карцинома кишечника CT26 CT26 colon tumor	Липосомальная форма фталоцианина цинка Liposomal zinc phthalo-cyanine	1,1 МГц, 1 Вт/см^2, 10 мин; 300 Дж/см^2, 160 мВт/см^2, λ=670±20 нм 1.1 MHz, 1 W/cm^2, 10 minutes; 300 J/cm^2, 160 mW/cm^2, λ=670±20 nm	В группах животных, получавших лечение методом СФДТ, отмечено статистически значимое торможение роста опухолей (p<0,01) и улучшение показателей выживаемости (p<0,05) Statistically significant inhibition of tumor growth (p<0.01) and improvement in survival rates (p<0.05) were observed in groups of animals treated with the SPDT method.
Wang P. et al., 2015 [36]	Адено-карцинома молочной железы 4T1 Адено-карцинома молочной железы человека MDA-MB-231 Адено-карцинома молочной железы человека MCF-7 Murine 4T1 mammary cancer Human breast cancer MDA-MB-231 Human breast cancer MCF-7	Хлорин e_6, 1 мкг/мл Chlorin e_6, 1 mkg/ml	1 МГц, 0,36 Вт/см^2, 1 мин; 1,2 Дж/см^2, λ=650 нм 1 MHz, 0.36 W/cm^2 1 minute; 1.2 J/cm^2 λ=650 nm	Коэффициент торможения роста опухоли на 22-е сутки: ФС + ФО – 24,22%; ФС + УЗ – 25,87%; ФС + ФО + УЗ – 47,48%; ФС + УЗ + ФО – 52,2% (p<0,01 – контроль; p<0,05). Среднее число метастазов в группах: контроль – 63,43; ФС + ФО – 39,14; ФС + УЗ – 38,43; ФС + ФО + УЗ – 16,43; ФС + УЗ + ФО – 24,43. The rate of tumor growth inhibition on the 22nd day after treatment: PS + PI – 24,22%; PS + US – 25,87%; PS + PI + US – 47.48%; PS + US + PI – 52.2% (p<0.01). Mean amount of metastasis in groups: control – 63.43; PS + PI – 39.14; PS + US – 38.43; PS + PI + US – 16.43; PS + US + PI – 24.43.

Tomankova K. et al., 2014 [37]	Карцинома шейки матки HeLa Cervical carcinoma HeLa	Дисульфонат фталоцианина хлоралюминия ClAlPcS2, 0,5; 5; 50 мкг/мл Chloroaluminium pthalocyanine disulfonate, 0.5, 5 and 50 mkg/ml	1 МГц, 2 Вт/см^2, 10 мин; 7,2 Дж/см^2, λ=660 нм 1 MHz, 2 W/cm^2, 10 minutes; 7.2 J/cm^2, λ=660 nm	Авторами отмечено статистически значимое увеличение количества апоптотических и некротических клеток в группе ФС + УЗ + ФО по сравнению с ФС + УЗ и ФС + ФО (p<0,001). The authors noted a statistically significant increase in the number of apoptotic and necrotic cells in the PS + US + PI group compared with PS + US and PS + PI (p<0.001).
Li Q. et al., 2014 [38]	Рак молочной железы мышей 4T1 Murine 4T1 mammary cancer	Хлорин e$_6$, 1 мкг/мл Chlorin e$_6$, 1 mkg/ml	1 МГц, 0,36 Вт/см^2, 1 мин; 1,2 Дж/см^2, λ=650 нм 1 MHz, 0.36 W/cm^2, 1 minute; 1.2 J/cm^2, λ=650 nm	Количество жизнеспособныхклеток: ФС – 101,52%;УЗ – 99,41%; ФО – 101,84%;УЗ + ФО – 100,77%. ФС + УЗ – 85,6%, ФС + ФО – 69,11%, ФС + УЗ + ФО – 47,8% (p<0,01). Активные формы O$_2$:контроль – 5,87%; ФС + УЗ – 7,77%;ФС + ФО – 62,93%; ФС + УЗ + ФО – 83,83%. Amount of viable cells: PS – 101.52%,US – 99.41%, PI – 101.84%, US + PI – 100.77%, PS + US – 85.6%, PS + PI – 69.11%, PS + US + PI – 47.8% (p<0.01) Reactive oxygen species: control – 5.87%, PS + US – 7.77%, PS + PI – 62.93%, PS + US + PI – 83.83%.
Wang H. et al., 2013 [39]	Адено-карцинома молочной железы человека MDA-MB-231 Human breast cancer MDA-MB-231	Хлорин e$_6$, 1 мкг/мл Chlorin e$_6$, 1 mkg/ml	1 МГц, 0,36 Вт/см^2, 1 мин; 1,2 Дж/см^2, λ=650 нм 1 MHz, 0.36 W/cm^2, 1 minute; 1.2 J/cm^2, λ=650 nm	Количество жизнеспособныхклеток: ФС – 100,35%; УЗ – 99,41%; ФО – 102,08%; УЗ + ФО – 103,83%. ФС + УЗ – 90,21% (p>0,05); ФС + ФО – 74,4% (p<0,05); ФС + УЗ + ФО – 45,08%; ФС + ФО + УЗ – 51,2%. Amount of viable cells: PS – 100.35%, US – 99.41%, PI – 102.08%, US + PI – 103.83%, PS + PI – 74.4% (p>0.05), PS + US + PI – 45.08%, PS + PI + US – 51.2% (p<0.05).

Li J.H. et al. 2013 [40]	Глиома С6 Glioma C6	Гемато-порфирин, 10 мкг/мл Hematoporphyrin, 10 mkg/ml	1 МГц, 0,5 Вт/см^2, 1,5 мин; 20–240 Дж/см^2, λ=630 нм 1 MHz, 0.5 W/cm^2, 1.5 minutes; 20–240 J/cm^2, λ=630 nm	Авторами отмечено статистически значимое увеличение количества апоптотических и некротических клеток, активных форм O_2 в группе ФС + УЗ + ФО по сравнению с ФС + УЗ и ФС + ФО (p<0,001). Максимальная инициация апоптоза отмечена при комбинации УЗ и ФО в дозе 80 Дж/см^2. The authors noted a statistically significant increase in the number of apoptotic and necrotic cells, reactive oxygen species in the PS+US+PI group compared with PS + US and PS + PI (p<0.001). The maximum initiation of apoptosis was observed with a combination of US and PI at an exposure dose of 80 J/cm^2.
			In vivo	
Wang P. et al., 2015 [36]	Адено-карцинома молочной железы мышей 4T1 Мыши BALB/c Murine 4T1 mammary cancer, BALB/c mice	Хлорин e_6, 20 мг/кг Chlorin e_6, 20 mg/kg	1,9 МГц, 1,6 Вт/см^2, 3 мин; 120 Дж/см^2, λ=660 нм 1.9 MHz, 1.6 W/cm^2, 3 minutes; 120 J/cm^2, λ=660 nm	Количество жизнеспособных клеток через 24 ч после лечения: контроль и ФС + УЗ – без эффекта; ФС + ФО – 30,89%; ФС + УЗ + ФО – 52,17%; ФС + ФО + УЗ – 55,71% (p<0,05). Amount of viable cells 24 hours after treatment: control and PS + US – without effect: PS + PI – 30.89%, PS + US + PI – 52.17%, PS + PI + US – 55.71% (p<0.05).
Церковс-кий Д.А. и др., 2015 [41, 42]	Глиома С6 Крысы Glioma C6 Rats	Фотолон, 2,5 мг/кг Photolon, 2.5 mg/kg	0,88 МГц, 0,7 Вт/см^2, 10 мин; 50 Дж/5 мм^2, λ=660±5 нм 0.88 MHz, 0.7 W/cm^2, 10 minutes; 50 J/5 mm^2, λ=660±5 nm	Увеличение продолжительности жизни животных по отношению к контролю для групп: операция + ФС + УЗ – 88,1%; операция + ФС + ФО – 122,4%; операция + ФС + УЗ + ФО – 194,1% (p<0,05). Increase of life expectancy for animals compared to the control for groups: surgery + PS + US – 88.1%, surgery + PS + PI – 122.4%, surgery + PS + US + PI – 194.1% (p<0.05).

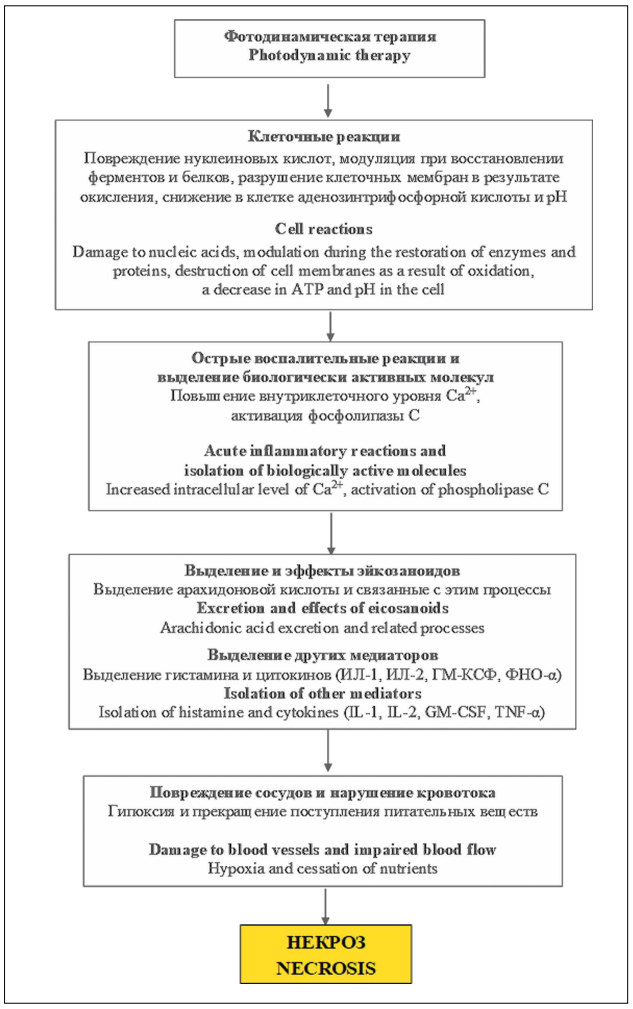

Рис. 1. Механизм некроза при ФДТ (Goldman M.P., 2010)
Fig. 1. PDT-induced necrosis (Goldman M.P., 2010)

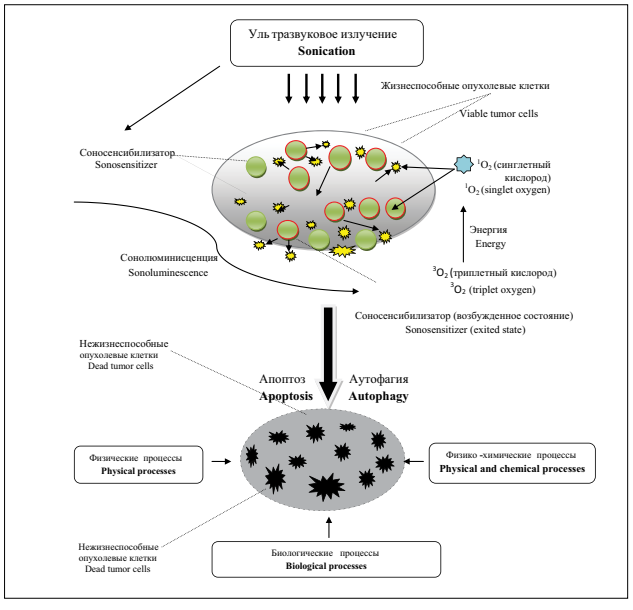

Рис. 2. Механизмы, лежащие в основе сонодинамической терапии
Fig. 2. Mechanisms of sonodynamic therapy

the SPDT method with the Sonoflora 1 PS (chlorophyll derivative, 30–60 mg, subglossally, 2–3 days). Photoradiation was carried out in a low-intensity mode using laser equipment with a radiation wavelength of 630 nm (light dose was 36 J/cm^2; power density was 20 mW/cm^2; duration was 30 min), ultrasonic treatment was conducted with a frequency of 1 MHz and an intensity of 2 W/cm^2 for 20 minutes. The therapy began 3–4 days after the PS administration and lasted 3 days. The treatment was repeated every 1–2 weeks.

Patient 1 had the progressive breast carcinoma after surgical treatment, chemoradiation therapy, Herceptin, Zometa courses, etc. After 3 SDT sessions, the relief of clinical symptomatology and the partial response were detected (according to the data from PET/CT). Patients 2 and 3 had the progressive breast carcinoma with metastatic lesions appearing in internal organs. After 2 SDT sessions, the partial response were reported (according to the data from PET/CT). 28 months after the treatment, distant metastases were not detected [50, 51].

L. Q. Li et al. presented the first case of using SPDT with the Sonoflora PS (subglossally, on the 1st and 2nd day) in 7 patients with esophageal and gastric adenocarcinomas at the ASCO Annual Meeting in 2014. From the 4th to the 6th day, both the tumor growth zone and the patient's entire body were exposed to the photoradiation and ultrasound. In 2 patients, no adverse reactions were noticed. In 5 patients, there were adverse events of the 1st and the 2nd grade (moderate pain, burns), which were easily relieved. The complete regression rate was 42.8% (n=3), the partial regression rate was 42.8% (n=3), and no effect was in 1 patient. The objective therapeutic effect was 85% [49].

D. Murphy et al. from the Skills Laboratory RACS (Melbourne, Australia) presented the results of the phase one clinical trial using SPDT with the Radochlorin, Sonnelux and Photosoft photosensitizers in the treatment of 66 patients with prostate cancer after the curative surgical treatment. The photosensitizers were administered subglossally or orally 16–24 hours before the treatment. Photoradiation parameters: the maximum power was 2 W, the absorbed dose of light was 4000-5000 J. Ultrasound parameters: 1 W. The treatment was conducted through transrectal, transurethral and percutaneous approaches. The maximum session duration was 25 minutes. The course of treatment included 3 procedures per week and was repeated twice for 12 months. The morbidity was 1.5% (n=1 is an urethral stricture). The authors noted the relief of clinical symptoms of the disease, the stabilisation or reduction of PSA after 6 months, the decrease in prostate volume and the absence of erectile dysfunction [52].

In 2009, J. N. Kenyon from The Dove Clinic (Hampshire, England) published the outcomes of treatment of 115 patients with malignant tumors (n=31, breast cancer; n=14, inoperable lung cancer; n=13, colon cancer; n=8,

prostate cancer; n=6, ovarian cancer; n=6, lymphoma; n=4, head and neck tumors; n=4, esophageal cancer; n=3, cervical cancer, n=3, gliomas, etc.) using SPDT with the Sonnelux-1 metallo-chlorine agent as a PS. The sublingual administration of the PS was slow and lasted for 2–5 hours. Photoradiation was performed using lasers with radiation wavelengths of 660 nm and 940±30 nm, ultrasonic treatment was conducted with a pulse intensity of 1 W/cm^2. The course of treatment included 3 sessions. The authors noted the high tolerability of the method and the absence of serious adverse events. The detailed description of findings upon survival criteria can be found in the publication [33].

Zhang W. et al. published the results of the pilot study involving the use of SPDT combined with chemotherapy in the treatment of 12 patients with metastatic (brain, internal organs, bones) breast cancer. SF1, SFa and UF chlorophyll derivatives were used as photosensitizing agents. Ultrasonic treatment was conducted both in continuous and pulsed modes with a pulse intensity of 1±10% MHz and an intensity of 2 W/cm^2 for 20–40 minutes daily 4–6 days after the sublingual administration of the PS and immersion of patients into a special water bath. The radiation was supplied to both nidi and the patient's entire body from 125 specially designed ultrasonic applicators. Photoradiation was carried out using laser equipment in a low-intensity mode (λ=554 nm, 45 mW/cm^2) for 30 minutes daily. 9 of 12 patients received additional chemotherapy treatment (according to standards of treatment accepted in the research centre). The number of SPDT courses was 1–4: 3 courses of SPDT in the mono mode, 9 courses of SPDT + chemotherapy. The median follow-up time was 34 months (9–68). Detected adverse events corresponded to grades 1-3 (CTCAE, version 3.0): weakness, pain in the nidus area, etc.). The therapeutic response was observed in 75% of cases, the complete regression rate was 16.7%, the partial regression rate was 58.3%, and the stabilisation was observed in 25% of cases. The authors concluded that the inclusion of SPDT in the comprehensive treatment regimen for patients with metastatic breast cancer can improve the outcomes of treatment of this severe disease and expand the range of therapeutic options [53].

During the study in our clinic, we tested the method of intraoperative SPDT with the chlorine based PS in 15 patients with recurrent glioblastoma. Methodology: the photolon solution was administered to patients via IV infusion at a dose of 2–2.5 mg/kg 30 minutes before the end of the surgery in the form of total or near-total resection of the recurrent tumor. After the infusion, the tumor bed was filled with 0.9% saline solution, then local ultrasonic treatment was conducted with a pulse rate of 1 MHz and a radiation intensity of 1 W/cm^2 for 10 minutes (Phyaction USTH 91, GymnaUniphy N.V., Bilzen). At the second stage after meticulous hemostasis, photoradiation of the tumor bed and walls was performed at light doses of 50–100 J/cm^2 using a laser apparatus generating radiation with a wavelength of 660±5 nm (UPL-FDT, Imaf Axicon, Belarus). The adverse reaction rate was 20% (n=2, convulsive disorder; n=1, cerebral edema with the development of hemiparesis and paresthesias). All adverse events corresponded to grades 1-2 of the CTCAE criteria (Version 4.0), were easily relieved and were not directly related to the sono-photodynamic therapy. The median overall survival of deceased patients was 23.1 months; the median survival after SPDT was 8.2 months [54].

Conclusion

As indicated by results of the experimental studies in cell culture and laboratory tumor-bearing animals presented in the literature, SPDT is an efficient option for antitumor treatment of various specific forms of malignant tumors [35-42]. Currently, several research teams are taking the first steps in testing the method in a clinical setting. The scientists from the South-East Asia presented preliminary results of using SPDT with photosensitizing agents in the treatment of malignant lesions of the breast, stomach, esophagus, prostate, lung and brain. The analysis of the obtained data indicates the absence of serious adverse events and increase in antitumor effects of the treatment regimens that have included SPDT with chlorine based photosensitizers [48–54].

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To the 115th anniversary of Russian radiology

THE HISTORY OF THE DEVELOPMENT OF RADIATION THERAPY: RADIATION DIAGNOSIS IN THE MRRC THEM. A.F. TSYBA

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In 2018, Russian radiology celebrated its 115th anniversary. The history began in 1903, along with the foundation of first Russian Department of Radiation Oncology at the Institute named after Morozov (now P. Hertsen Moscow Oncology Research Center). For this event, staff of the FSBI NMRRC of the Ministry of Health of the Russian Federation and a number of other healthcare organizations prepared a series of op-ed articles on the development and success of the clinical use of radiology in our country.

In this article, the authors propose to get acquainted with the history of the development of ray diagnostics in one of the largest Russian radiological centers – A. TSYB MRRC. Since the foundation of the Institute of Medical Radiology, USSR Academy of Medical Sciences (IMR AMS USSR), academician G.A. Zedgenidze organized a ray diagnostics service, that was one of the most advanced in those times in our country, both in terms of equipment and qualifications of experts. Currently, Ray Diagnostics Department at A. Tsyb MRRC continues to bear a banner of one of the best in Russia. Modern diagnostic tools installed in the departments allow to detect neoplasms in the initial stage of development, which, on the one hand, ensures high response to the therapy and relapse-free survival, and on the other hand, diagnosis of cases of negative changes of course of disease. In many respects, thanks to the high qualification and well-coordinated work of the team of doctors, medical scientists from clinical and experimental sectors of the Center, the existing methods are improved, new recommendations and protocols of ray diagnostics research are being developed and actively implemented.

"The scientist's vision is limited to their instruments."
Niels Bohr

К 115-летию отечественной радиологии

ИСТОРИЯ РАЗВИТИЯ ЛУЧЕВОЙ ТЕРАПИИ: ЛУЧЕВАЯ ДИАГНОСТИКА В МРНЦ ИМ. А.Ф. ЦЫБА

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Ray diagnostics is one of the main and most frequently used methods for oncology diseases diagnosis. The existence of cancer service and its further development without diagnostic radiology methods is impossible. Radiological methods are an important link in modern anticancer therapy and are used to determine neoplasm localization, its stage, and also to monitor response to the treatment. In other words, the examination of patient as they admit the medical institution begins with ray diagnostics and, based on the results of imaging, the decision on discharge is made.

The Institute of Medical Radiology, USSR Academy of Medical Sciences (IMR AMS USSR) was created as the largest oncological institute. 1962 is considered to be the year of the institute foundation, since it was precisely that year that the Order of the Minister of Health of the USSR of September 1 on the organization of the Institute of Medical Radiology was issued, and the construction of the experimental sector building was completed. However, the Decree of the CPSU Central Committee and the Council of Ministers of the USSR on the construction of the institute was adopted in August 1958, at the same time the Soviet radiologist, academician of AMS USSR (1960) Georgy Artemyevich Zedgenidze, was appointed to director of the future Institute of Medical Radiology. In 1970, the Institute was renamed to the Scientific Research Institute of Medical Radiology, and since 1992, the Medical Radiological Research Center. Today it is A.F. Tsyb Medical Radiological Research Center, which since 2014 is a branch National Medical Research Radiological Center of the Ministry of Health of the Russian Federation (as amended by the Ministry of Health of the Russian Federation Order of July 12, 2017 No. 427).

In the introduction of the textbook for students of medical universities "On teaching radiology in medical institutes" written by G.A. Zedgenidze in collaboration with L.D. Lindenbraten, the authors note that "... the textbook is made up taking into account the current level of development of science and medical practice..." The introduction states that medical radiology is divided into four main sections: 1) general radiology; 2) X-ray diagnosis; 3) radiologic diagnosis; 4) radiation therapy (radiotherapy). In accordance with them, Department of Radiology in the clinical sector of IMR USSR was created, which included X-ray Diagnosis Department and Radiologic Diagnosis Department. Department of Radiology was headed by G.A. Zedgenidze, and Radiologic Diagnosis Department – M.N. Fateeva.

Over time, medical diagnostic equipment was improved and the Center installed new equipment, which was based on modern physical methods of imaging, therefore, since 2006, Department of Radiology has been expanded and renamed to Ray Diagnostics Department (Head is Z.N. Shavladze, Candidate of Medical Science). The department has the following departments: X-ray diagnosis, ultrasonic diagnosis (Professor V.S. Parshin), radionuclide diagnostics (G. A. Davydov, Candidate of Medical Science), computer-assisted diagnosis (N.K. Silantyeva, Doctor of Medical Science) and MR imaging (Z.N. Shavladze, Candidate of Medical Science).

However, despite the rapid development of modern methods of ray diagnostics, classical X-ray apparatus have the most widespread use. Traditional X-ray radiography remains one of the popular methods, as before, although more than 100 years have passed since its first use.

Since the foundation of the Institute, X-ray Diagnosis Department has been part of the staff of the Department of Radiology. From the very beginning of its organization, the work was divided into several directions. Responsibility for X-ray diagnosis of respiratory diseases was assigned to I.S. Amosov, with the direct participation of whom X-ray stand "Rentpolygraph VRP-2/4" , designed to study the function of the external respiration of a man, was developed. Thanks to I.S. Amosov works, X-ray methods for studying lungs and heart functional status were created, methods of X-ray pneumopolygraphy and X-ray tetragraphy, which allow to simultaneously study both lung tissue respiratory capacity and contribution of tidal volumes to the gas exchange process, were developed and implemented. The description of the developed methods of X-ray diagnosis has been repeatedly published in scientific journals and guidelines. The study of pathologies of the gastrointestinal tract was headed by P.V. Vlasov – co-author of the monograph "Relief of the gastric mucosa in health and disease." Responsibility for the study of the musculoskeletal system was assigned to M.D. Saidov, who published scientific articles on X-ray diagnosis of bone neoplasms and congenital skeletal system defects throughout his short work period. X-ray examinations of urological and gynecological diseases were V.A. Kulikov prerogative. He is a co-author of monographs and guidelines, the most important of which are: "X-ray examination

of laboratory animals" , "Ray diagnostics and radiation therapy of bladder cancer" , "Intravenous angiography in kidney disease diagnosis, accompanied by hypertension" , "Vesiculography for prostate cancer."

In 1973, V.A. Kulikov was appointed to the Head of X-ray Diagnosis Department, and I.S. Amosov became the Head of the department.

A significant contribution to organization of Ray Diagnostics Department, its offices, provision of departments with medical equipment, development and introduction of new methods of X-ray examination of organs and systems were made by professors I.S. Amosov, V.A. Kulikov, P.V. Vlasov, P.M. Kotlyarov; Doctors of Medical Science Yu.G. Elashov, B.M. Astapov, V.A. Degtyarev, P.V. Zharkov; Candidates of Medical Science N.V. Afanasova, Yu.N. Konstantinov.

As remembered by the Center staff, Yu. G. Elashov, Doctor of Medical Science, helped employees of different departments, not only radiologists, but also applicants from the union republics in preparing and defending dissertations during all the years of work at the Institute.

Under V.A. Kulikov, The staff of X-ray Diagnosis Department issued more than 120 scientific publications, including 2 monographs (in co-authorship), obtained 2 patents for inventions. He was a dissertation advisor of 7 PhD theses, that were defended.

Throughout the history of MRRC development, there is a constant improvement in the existing methods and introduction of new methods and devices for ray diagnostics, which significantly expands the possibilities of imaging. Today, one of the main tasks of diagnostic radiology is the introduction of modern diagnostic methods and the timely updating of medical equipment fleet.

Since 90s a new stage is beginning at the Medical Radiological Research Center, connected with the introduction of modern imaging methods. Under the Center modernization programme, X-ray computed tomography (CT) equipment was purchased and in 1991 a same-name department was organized, which was headed by N.K. Silantyeva, Candidate of Medical Science. Computed Tomography Department is actively involved in scientific work, scientific articles and monographs are published.

In 2006, a Magnetic Resonance Imaging (MRI) Department was organized in the Center under the supervision of Z.N. Shavladze, Candidate of Medical Science. Professor T.P. Berezovskaya, Doctor of Medical Science, has been working in the Department since its foundation. Under T.P. Berezovskaya, minimally invasive therapeutic and diagnostic interventions are being actively implemented under the control of computed tomography (CT) and MRI, MR-angiography sect is being developed.

The introduction and widespread use of modern imaging methods such as CT and MRI has expanded the possibilities of oncology diseases verification.

Radionuclide Diagnostics Department is of particular historic importance in the activities of the entire Center. Initially, the department was created as a laboratory of radiologic research methods within Radiologic Diagnosis Department, founded in 1962. The first Head of Radiologic Diagnosis Department was Professor M.N. Fateeva, who stood at the very beginning of Russian radionuclide diagnostics. Later, from 1969 to 1976, the department was headed by Professor R.I. Gabunia; from 1976 to 1998 – Professor E.G. Matveenko, and in 1998–2005. – G.A. Davydov, Candidate of Medical Science. In 2005, the laboratory of radiologic research methods became part of Ray Diagnostics Department and was renamed to Radionuclide Diagnostics Department, which has been headed by G.A. Davydov until now.

Professor M.N. Fateeva and her students gave a theoretical justification for the principles and methods of radionuclide diagnostics in various fields of medicine: endocrinology, oncology, gastroenterology, cardiology, pneumology, uronephrology, osteology and others, which were introduced into clinical practice along with the expansion of radioisotope diagnostics laboratories network in the country.

An important feature radionuclide diagnostics development in our Center is the constant conduct of experimental and clinical research. Thanks to the activities of the department, more than 60 methods of radionuclide diagnostics were developed and improved, for the introduction into clinical practice of which 50 medals of VDNKh (All-Union Exhibition of Achievements of National Economy) USSR were received, including 6 gold, 9 silver and 35 bronze. The results of scientific research papers of the department staff are summarized and published in the form of 13 monographs, 7 collections, 25 guidelines and over 500 articles, 47 invention certificates were received. Over the entire period of department's activities, 10 doctors and 35 candidates of science were trained, many of whom became heads of institutes and laboratories in Russia and CIS countries.

In recent years, employees have published more than 100 scientific articles, participated in writing of several monographs and manuals.

Ultrasonic Diagnosis Department was founded in 1988 on the basis of existing Lymphangiography Department due to the fact that by that time ultrasound tomographs made up a body of the diagnostic equipment. In turn, the history of Lymphangiography Department began on the basis of the group of lymphangiography, which was organized and successfully supervised by A.F. Tsyb. In Lymphangiography Department, X-ray semiotics of neoplasms was and the processes of recurrence and progression of malignant tumors after antineoplastic therapy were studied, and the morphological and functional changes that occurred after radiation and combined therapy of cancer were determined. Methods for the study of lymphatic system using various diagnostic techniques were developed.

A temporary international team of countries of the Council for Mutual Economic Assistance on Lymphangiography was formed and effectively worked on the basis of the department; methods and devices for conducting lymphography were developed. Fundamental X-ray findings on the anatomy and function of thoracic duct and other parts of the lymphatic system were obtained. The technique of total intravital microangiography was developed.

The following doctors successfully worked in the department: B.Ya. Drozdovsky, O.V. Nestayko, G.V. Chepelenko, A.P. Kislitsyn, V.I. Strigunov, V.V. Yarzutkin, O.N. Ostapovich, I.Kh. Mukhamedzhanov, A.I. Dergachev, V.S. Parshin and G.N. Grishin.

From 1988 to 2006, the former Director of MRRC, Academician A.F. Tsyb, was the eternal Head of Ultrasonic Diagnosis Department. He is the author and co-author of over 500 scientific works, including 26 monographs. The most important diagnostic works are the following: "Clinical lymphography" , "Diagnosis and combined treatment of rectal cancer" , "Guidelines for ultrasonic diagnosis of diseases of abdominal organs and retroperitoneal space" , "Ultrasonic tomography and targeted biopsy in diagnosis of pelvic tumors." Under A.F. Tsyb, The department strengthened and developed, new ultrasonic apparatus were purchased. The era of ultrasonic diagnosis has begun at the Center. First doctors worked at the department were A.I. Dergachev, V.S. Parshin, I.Kh. Mukhamedzhanov, G.N. Grishin, S.G. Shakhova.

After technogenic disaster at the Chernobyl Nuclear Power Plant and release of radioiodine into the atmosphere, it became necessary to carry out a screening survey of the population living in contaminated areas. Over the 20-year period, more than 250,000 ultrasonic examinations of thyroid gland were performed, and an organ screening technology for early diagnosis was developed. According to the results of the surveys, many scientific articles and monographs were published, the most important of which is "Thyroid cancer. Ultrasonic diagnosis. Clinical Atlas. On the basis of Chernobyl materials." The research results of the department were included in 2 reports that were presented to the United Nations General Assembly on the medical consequences of Chernobyl accident.

In 2006, Ultrasonic Diagnosis Department was headed by Professor V.S. Parshin. On the basis of the department, 7 doctors and 32 candidates of medical science were trained, 21 monographs were published.

At Ultrasonic Diagnosis Department, in addition to the main work, screening surveys of the population of the Bryansk and Kaluga regions continue under the Government Contract of the Center with the Emergency Control Ministry "The introduction of advanced medical technologies in the diagnosis and treatment of citizens with cancer" and "Screening technology for thyroid gland malignant diseases diagnosis." To enhance practical significance of studies and share experience, the department works closely with doctors from Japanese universities in Nagasaki and Fukushima.

Department of Laser and Photodynamic therapy was founded in 1998. The Head of the department since its first day until now is Professor M.A. Kaplan, Doctor of Medical Science. Under Professor Kaplan, work is underway to study the impact of laser irradiation on biological objects, the development of new laser therapy equipment and photosensitizers for photodynamic therapy, the creation and subsequent introduction into clinical practice of new methods of laser irradiation. A new sect of laser medicine is being developed in the department – photodynamic therapy and fluorescent diagnostics, which are effectively used to diagnose and image foci of skin neoplasms and mucous membranes. The use of domestic alasens and photolon photosensitizers allows the use of the following test – fluorescence spectroscopy with additional imaging of skin tumor lesions. Present test helps to determine boundaries of tumor lesion, which makes it possible to conduct a more definitive course of treatment and reduce the number of so-called marginal recurrences. In the process of diagnostics, it is possible to reveal hidden lesions, as well as monitor the content of medication in skin, the course of the treatment and assess the duration of skin phototoxicity period. The work of the department is carried out in clinical and experimental sects, according to its results, methods of treatment and diagnosis of skin and mucous membranes masses have been developed. The department works closely with the Kaluga branch of S.N. Fedorov NMRC MNTK "Eye Microsurgery" in the field of of development of fluorescent diagnostics and therapy methods in ophthalmology. In addition, photodynamic therapy is used in the treatment of cancer of oral musoca, lower lip, lung, esophagus, stomach and vulva.

In 2018 at A. Tsyb MRRC, Department of X-ray Surgical Diagnostic and Treatment Methods was opened under the supervision of V.V. Kucherov, Candidate of Medical Science. Chemoembolization and radioembolization of liver, unique surgeries for Russia, are performed here. Radioembolization in the treatment of liver cancer and metastases is carried out using domestic microspheres based on yttrium-90 radionuclide.

The introduction of MRRC into the united Center represented by FSBI NMRRC of the Ministry of Health of the Russian Federation expanded the possibilities of introducing into our clinical practice the results of our basic research, opening up new ways to successfully sort out current problems of development and high-quality application of new methods of therapeutic radiology.

The improvement of existing methods, the conduct of experimental and scientific research within the walls of MRRC that promote the introduction of new protocols of ray diagnostics into clinical practice, reinforce the significance and effectiveness of diagnostic radiology in cancer service.

PRESS RELEASE of the V INTERNATIONAL WINTER SCHOOL "PHOTODYNAMIC THERAPY AND PHOTODIAGNOSTICS"

From 11 to 15 February 2019, the V International Winter School, traditionally devoted to the use of photodynamic therapy (PDT) and fluorescent diagnostics (FD) in practice, was held on the basis of P. Hertsen Moscow Oncology Research Center and Prokhorov GPI RAS. The school was organized by Russian Photodynamic Association, FSBI NMRRC of the Ministry of Health of the Russian Federation, FSAEI HE National Research Nuclear University MEPhI and Federal Publicly Funded Institution of Science Prokhorov GPI RAS.

The winter school on fluorescent diagnostics and photodynamic therapy has widely established itself as a research and practice event for students, postgraduates, research scientists, physicists and medical specialists working in the field of medical photonics and who want to improve their knowledge and practical skills.

In 2019, more than 80 specialists from the Central (Moscow, Obninsk, Tula) and the North Caucasus (Makhachkala) federal districts of the Russian Federation took part in the winter school. Among the participants were representatives of the following scientific areas: oncology, surgery, dermatology, urology, endoscopy, pharmacology, biology, biophysics, biochemistry, veterinary science. Specialists from practicing medical organizations (clinical oncology dispensary of Moscow and Tula, the Republican Clinical Hospital of Makhachkala, the Republic of Dagestan, State Budgetary Healthcare Institution Family Planning and Reproductive Moscow Health Department, Clinic of Modern Medical Technologies (KST-group), State Budgetary Healthcare Institution The Moscow State Public Health Institution of Health Moscow Scientific and Practical Center for Dermatovenerology and Cosmetology of Moscow City Health Department; scientific research institutes (P. Hertsen Moscow Oncology Research Center and A.F. Tsyb MRRC – branches of FSBI NMRRC of the Ministry of Health of the Russian Federation, NIOPIK SSC FSUE, National Research Nuclear University MEPhI, Prokhorov GPI RAS); business (LLC "Veta-Grand", LLC "Company OPT-MMOL") and educational centers (Pirogov Russian National Research Medical University, FSAEI HE National Research Nuclear University MEPhI).

The program of the winter school consisted of lectures and seminars. Part of the classes was conducted in a joint mode, for medical specialists and physicists the other part was divided by specialties.

The program of the school's lecture sessions a wide range of issues was discussed, including analyzing the market for Russian photosensitizers, developing and testing new photosensitizers (including from those made of natural raw materials), instruments and tools for conducting PDT and FD. Special attention was paid to modern development directions and the latest achievements of PDT and FD, as well as promising approaches in clinical practice. Lectures were given by leading Russian and foreign experts in the field of photodynamic therapy and photodiagnostics. Among others the following messages were read: "Russian photosensitizers in clinical practice", "Targeted photosensitizers based on porphyrins, chlorins and their metal complexes", "Photosensitizers based on natural pigments", etc. Some reports were presented in English ("Immunological aspects of PDT"). Upon registration all participants were provided with the materials, including guide on photodynamic therapy.

In the framework of skills building session, students were introduced to device and operating principles of the equipment for FD and PDT of various localizations, with the peculiarities of their use in laboratory and clinical conditions. Specialists had the opportunity to get acquainted with the methodology of fluorescence microscopy, as well as work of a video fluorescence analyzer, installations for determining the concentration of photosensitizer in vivo, and other equipment. Also, seminars included the analysis of private clinical cases of the use of PDT and FD in practice.

During breaks students of the school had the opportunity to ask lecturers and class leaders questions of their interest, clarify technical details and features of the use of specific photosensitizers, devices and methods.

Listeners noted the high level of reports and skills building session at the School. A large number of questions asked and the interest shown to the event reflect the relevance and practical significance of conducting such educational programs.