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

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

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ORIGINAL ARTICLES

ОРИГИНАЛЬНЫЕ СТАТЬИ

REVIEWS OF LITERATURE

ОБЗОРЫ ЛИТЕРАТУРЫ

ORIGINAL ARTICLES

INFLUENCE OF PHOTODYNAMIC THERAPY ON THE LEVEL OF MATRIX METALLOPROTEINASES IN SQUAMOUS CELL SKIN CANCER

Maslyakov V.V.[1], Grebnev D.Yu.[2], Tsymbal A.A.[3], Kim L.M.[4]
[1]Federal State Budgetary Educational Institution of Higher Education «Mari State University», Yoshkar-Ola, Russia
[2]Federal State Budgetary Educational Institution of Higher Education «Ural State Medical University», Yekaterinburg, Russia
[3]First Moscow State Medical University named after I.M. Sechenov, Moscow, Russia
[4]Private institution educational organization of higher education «Medical University «Reaviz»», Saratov, Russia

Abstract

The effect of photodynamic therapy (PDT) on the level of matrix metalloproteinases in squamous cell skin cancer (SCSC) was studied. The study involved 202 people, including 185 patients with SCSC, who were on outpatient and inpatient treatment in medical institutions of Engels and Saratov during the period from 2015 to 2019, and 17 donors. The study design included studies in three main groups. The first (intervention) group included 74 (36.6%) patients with SCSC who underwent combined treatment, including PDT at the first stage and surgical treatment at the second stage. The second group consisted of 111 (55.0%) patients with SCSC who underwent only surgical treatment. The third group consisted of 17 (8.4%) relatively healthy volunteer donors, comparable in age and sex with the patients of the main group. As a result of the study, it was found that the level of metalloproteinase-1 inhibitor (TIMP-1) in the blood serum of patients with SCSC was reduced compared with physiologically normal indicators, which led to a statistically significant increase in matrix metalloproteinases (MMPs) MMPs-2, MMPs-7 and MMPs-9. Performing only surgical treatment for this pathology does not lead to a complete recovery of these indicators. However, the use of combined treatment including PDT showed a statistically significant increase in the amount of TIMP-1 before the start of surgical treatment, which naturally led to a decrease in MMPs-2, MMPs-7 and MMPs-9. Later, after excision of the tumor, the patients of this group had a complete normalization of TIMP-1, which, in turn, contributed to a decrease and then restoration of the number of MMPs-2, MMPs-7 and MMPs-9 to physiologically normal values.

Keywords: Squamous cell skin cancer, photodynamic therapy, metalloproteinases.

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Contacts: Maslyakov V.V., e-mail: maslyakov@inbox.ru

ВЛИЯНИЕ ФОТОДИНАМИЧЕСКОЙ ТЕРАПИИ НА УРОВЕНЬ МАТРИКСНЫХ МЕТАЛЛОПРОТЕИНАЗ ПРИ ПЛОСКОКЛЕТОЧНОМ РАКЕ КОЖИ

В.В. Масляков[1], Д.Ю. Гребнев[2], А.А. Цымбал[3], Л.М. Ким[4]
[1]ФГБОУ ВО «Марийский государственный университет», Йошкар-Ола, Россия
[2]ФГБОУ ВО «Уральский государственный медицинский университет», Екатеринбург, Россия
[3]ФГАОУ ВО Первый Московский государственный медицинский университет имени И.М. Сеченова, Москва, Россия
[4]Частное учреждение образовательная организация высшего образования «Медицинский университет «Реавиз», Саратов, Россия

Резюме

Изучено влияние фотодинамической терапии (ФДТ) на уровень матриксных металлопротеиназ при плоскоклеточном раке кожи (ПКРК). В исследовании участвовали 202 человека, из них 185 пациентов с ПКРК, находившихся на амбулаторном и стационарном лечении в лечебных учреждениях г. Энгельса и г. Саратова в период с 2015 по 2019 гг., и 17 доноров. Дизайн исследования включал в себя исследования в трех группах. В первую (основную) группу пациентов вошли 74 (36,6%) больных ПКРК, которым было проведено комбинированное лечение, включающее на первом этапе ФДТ, на втором – хирургическое лечение. Вторую группу составили

111 (55,0%) больных ПКРК, которым было выполнено только хирургическое лечение. Третья группа состояла из 17 (8,4%) относительно здоровых доноров-добровольцев, сопоставимых по возрасту и полу с пациентами основной группы. В результате проведенного исследования было установлено, что уровень ингибитора металлопротеиназы-1 (TIMP-1) в сыворотке крове у пациентов с ПКРК понижен по сравнению с физиологически нормальными показателями, что приводило к статистически достоверному повышению показателей матричных металлопротеиназ (MMPs): MMPs-2, MMPs-7 и MMPs-9. Выполнение только хирургического лечения при ПКРК не приводит к полному восстановлению данных показателей. Однако применение комбинированного лечения, включающего ФДТ, позволило уже до начала хирургического этапа статистически достоверно увеличить количество TIMP-1, что закономерно приводило к снижению уровня MMPs-2, MMPs-7 и MMPs-9. После иссечения опухоли у пациентов этой группы установлена полная нормализация TIMP-1, что, в свою очередь, способствовало снижению, а затем восстановлению количества MMPs-2, MMPs-7 и MMPs-9 до физиологически нормальных значений.

Ключевые слова: плоскоклеточный рак кожи, фотодинамическая терапия, металлопротеиназы.

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Контакты: Масляков В.В., e-mail: maslyakov@inbox.ru

Introduction

Squamous cell skin cancer (SCSC) accounts for about 20% of all malignant skin diseases [1, 2, 3]. SCSC is the most malignant epithelial tumor of the skin and mucous membranes with squamous differentiation that develops from keratinocytes [4]. SCSC is characterized by a destructive growth pattern with a gradual progression of the tumor process, infiltration of underlying tissues, metastasis to regional and distant lymph nodes (85%), hematogenous metastasis to internal organs (15%), such as lungs and bones, which can lead to the death of the patient [5]. Currently, various methods of treating this pathology have been proposed, including surgical resection of the tumor, radiation therapy, cryosurgery, laser excision, and the use of photodynamic therapy (PDT).

The PDT method is one of the modern methods of treatment in oncology. PDT is a minimally invasive and effective treatment for SCSC based on the use of photodynamic damage to tumor cells during photochemical reactions [6, 7]. After the introduction of a special substance, a photosensitizer, into the body, the photosensitized tissue is irradiated with a laser with a certain wavelength [8-10]. The results presented in the literature indicate that PDT is an effective organ-preserving method for the treatment of malignant neoplasms of the skin and mucous membranes, especially with unfavorable localization of neoplasms and in cases where the possibilities of traditional methods are limited. It has been established that complete resorption of the tumor can be achieved with a single or staged exposure without the development of side effects with maximum preservation of the viability of surrounding healthy tissues [1]. According to the literature, metalloproteinases (MMPs) are of great importance in the prognosis and mechanism of skin cancer development [11]. An analysis of the literature has shown that the effect of PDT on the change in the level of MMPs in SCSC is currently insufficiently studied. In the present study, the effect of PDT on changes in the level of MMPs in SCSC was studied.

Materials and methods

The study involved 202 people, of which 185 patients with SCSC were on outpatient and inpatient treatment in medical institutions in Engels and Saratov cities in the period from 2015 to 2019, and 17 healthy volunteers. Of the total number of patients, there were 129 (69.7%) men and 56 (30.3%) women. Based on the analysis of the localization of SCSC by anatomical regions, it was found that in 25% of cases the tumor was localized in the lower extremities, in the face – in 15%, in the region of various parts of the upper extremities – in 20%, in the neck – in 18% , in different parts of the chest – in 12% of cases, in 10% of cases, the tumor occupied various anatomical regions of the back. The diagnosis of SCSC was based on the collection of anamnestic data, complaints made by patients, and morphological examination. For morphological examination, a biopsy of the tumor tissue was performed, after receiving the result, the final diagnosis was established.

The research program included studies in three main groups. The first included 74 (36.6%) patients who underwent combined treatment: PDT and surgery, including wide excision of the tumor under intravenous anesthesia. The second group consisted of 111 (55.0%) patients with SCSC who underwent only surgical treatment. To control and compare the obtained laboratory parameters, a third group was created, which included 17 (8.4%) donors without established oncological pathology, who voluntarily agreed to participate in the study.

Patients included in group 1 had disease stage T1N0M0 in 25 (33.8%) cases, stage T2N0M0 – in 49 (66.2%) cases. In group 2, patients had disease stages T1N0M0 and T2N0M0 in 26 (23.4%) and 85 (76.6%) cases, respectively. Based on the analysis of the morphological findings, it was found that in 16% of cases, the spindle cell form of SCSC was noted, in the remaining 84%, the acantholytic form.

In all patients of groups 1 and 2, risk factors for de-

veloping cancer were identified in the analysis of anamnesis data (Table 1).

Comorbidity was detected in 98 (52.7%) patients with SCSC, including 43 (23.4%) patients in group 2, 55 (29.7%) patients in group 1, whose patients underwent preoperative PDT. The number of patients and the nature of comorbidities are presented in Table 2.

The study included patients with stage T1-2N0M0 SCSC. Patients with disseminated oncological process and patients who underwent radiation and/or chemotherapy were not included.

The studies were carried out after clarifying its purpose and objectives and obtaining permission from patients to participate in the study, which was confirmed by written consent. The study plan was heard at the local ethics committee of the Reaviz Medical University, where it received permission and approval (protocol No. 12 of November 16, 2020).

Treatment of patients in group 1 was carried out in several stages. At the first stage, PDT was performed. Photolon, manufactured by Belmedpreparaty (Republic of Belarus), was used as a photosensitizer. Photolon was

Таблица 1
Факторы риска развития рака у больных плоскоклеточным раком кожи
Table 1
Risk factors for cancer development in patients with squamous cell skin cancer

Факторы риска Risk factors	Число пациентов в группах Number of patients in groups			
	Группа 1 Group 1 (n = 74)		Группа 2 Group 2 (n = 111)	
	абс. число absolute number	%	абс. число absolute number	%
Воздействие вредных производственных факторов Exposure to occupational hazards	47	63,5	22	19,8
Генетическая предрасположенность Genetic predisposition	25	33,8	88	79,3
Курение табака Tobacco smoking	50	67,6	52	46,8

Таблица 2
Структура сопутствующей патологии у пациентов с плоскоклеточным раком кожи
Table 2
Structure of comorbidity in patients with squamous cell skin cancer

Нозологическая форма сопутствующей патологии Nosological form of concomitant pathology	Количество пациентов в группах Number of patients in groups	
	Группа 1 Group 1 (n = 74)	Группа 2 Group 2 (n = 111)
Артериальная гипертензия Arterial hypertension	12 (16,2%)	14 (12,6%)
Ишемическая болезнь сердца Coronary heart disease	11 (14,9%)	12 (10,8%)
Ожирение различной степени Various degrees of obesity	9 (12,2%)	11 (9,9%)
Эрозивный гастрит Erosive gastritis	16 (21,6%)	5 (4,5%)
Хронический холецистит Chronic cholecystitis	7 (9,4%)	1 (0,9%)
Всего Total	55 (74,3%)	43 (38,7%)

injected intravenously at a dose of 2.0 mg/kg of body weight in 200 ml of natural saline solution, the administration time took about 30 minutes. At the same time, laser irradiation of blood was performed using a krypton laser having a wavelength of 647-675 nm, with a power density of 120-300 mW. 3-4 hours after the end of intravenous injection, a session of local irradiation of the tumor was performed, for this, a Latus laser device (λ–662 nm, E = 300 J/cm2) was used. Performed 3 courses of PDT (introduction of a photosensitizer and irradiation) for 3 days. 5 days after the end of the third course of PDT, patients in this group underwent surgery.

In the course of the study, the levels of the inhibitor of metalloproteinase-1 (TIMP-1), matrix metalloproteinases (MMPs): MMPs-2, MMPs-7, MMPs-9 in the blood serum were determined before the start of PDT in group 1, in group 2 – before surgical removal of the tumor. Then these indicators were determined in group 1 on the fifth day after the end of PDT and before the surgical stage. In the postoperative period, in both groups, the sampling of biological material (blood plasma) was carried out on the first, third, fifth, seventh, tenth days and after 18 months. Blood sampling and analysis of indicators in the comparison group (group 3) was carried out once.

The level of TIMP-1 was determined by enzyme immunoassay using R&D Diagnostics Inc. (USA) reagents. This method determines the quantitative content of the test substance in a biological substrate by combining the substance with antibodies. Quantitative determination of MMPs in blood serum was performed using Human/Mouse/Rat (total) sera from Quantikine®, R&D Systems, which are standard and designed for direct enzyme immunoassay. For measurements, an automatic universal reader ELX800 from Bio-TekInstruments, Inc. (USA) was used, designed for microplates.

The results obtained during the study were subjected to mathematical processing on a personal computer with the Statistica 6.0 application package, as well as Excel. Hypotheses about the type of distributions were tested (Shapiro-Wilks test). Most of our data do not follow the normal distribution law, therefore, to compare the values, the Mann-Whitney U-test was used, on the basis of which the Z-test and the significance indicator p were calculated. The differences were considered statistically significant at $p < 0.05$. In addition, the Spearman rank correlation coefficient (R) and its reliability index p were calculated.

Results

It has been proven that in the process of carcinogenesis, including skin lesions, changes in the levels of TIMP-1 and MMPs occur [11]. In order to clarify the nature of the change in these indicators, the parameters were studied in all 185 patients who participated in the study, before the start of treatment (initial background) and during subsequent follow-up (see Table 3).

Before the start of treatment in both groups, there was a statistically significant decrease in the amount of TIMP-1 and an increase in the level of MMPs in the blood serum.

The use of PDT before performing surgical treatment for SCSC (group 1) leads to a statistically significant increase in the amount of TIMP-1 and a regular decrease in the concentration of MMPs-2, MMPs-7 and MMPs-9. This is confirmed by the fact that in the group of patients subjected to only surgical treatment, a statistically significant decrease in the TIMP-1 index was revealed, and, as a result, an increase in all MMPs indices in comparison with the results obtained in donors and patients who received combined treatment. Clearly, SCSC leads to changes in levels of TIMP-1 and MMPs-2, MMPs-7 and MMPs-9. There were no significant statistically significant differences in the studied parameters in patients with stage T1-2N0M0 SCSC.

On the first postoperative day in patients of group II, a statistically significant decrease in TIMP-1 to 436.4 ng/ml and an increase in all studied MMPs parameters were registered compared to the data obtained from donors.

Таблица 3
Динамика изменения уровня TIMP-1 и MMPs в сыворотке крови у пациентов с плоскоклеточным раком кожи после лечения
Table 3
Dynamics of changes in the level of TIMP-1 and MMPs in blood serum in patients with squamous cell skin cancer after treatment

Исследуемые показатели Researched indicators	Уровень TIMP-1 и MMPs, нг/мл Level of TIMP-1 and MMPs, ng/ml		
	группа 1 ФДТ + операция group 1 PDT + surgery (n=74)	группа 2 операция group 2 surgery (n=111)	группа 3 доноры group 3 donors (n=17)
до начала лечения (исходный фон) before treatment (initial background)			
TIMP-1	573 (526;742) $p_2 = 0,038$ $p_3 = 0,045$	567,3 (557;684) $p_1 = 0,032$ $p_3 = 0,025$	789 (771;793)

Исследуемые показатели Researched indicators	Уровень TIMP-1 и MMPs, нг/мл Level of TIMP-1 and MMPs, ng/ml		
	группа 1 ФДТ + операция group 1 PDT + surgery (n=74)	группа 2 операция group 2 surgery (n=111)	группа 3 доноры group 3 donors (n=17)
MMPs-2	612 (510;627) $p_2 = 0,032$ $p_3 = 0,045$	616 (598;627) $p_1 = 0,012$ $p_3 = 0,045$	254 (252;257)
MMPs-7	12,1 (9,3;14,7) $p_2 = 0,042$ $p_3 = 0,045$	10,4 (11,9;12,5) $p_1 = 0,018$ $p_3 = 0,045$	3,8 (3,6;4,0)
MMPs-9	852 (708;918) $p_2 = 0,013$ $p_3 = 0,056$	864 (840;910) $p_1 = 0,036$ $p_3 = 0,046$	396 (394;398)
после завершения ФДТ (группа 1) after completion of PDT (group 1)			
TIMP-1	679 (626;742) $p_2 = 0,038$ $p_3 = 0,045$	567,3 (557;684) $p_1 = 0,032$ $p_3 = 0,025$	789 (771;793)
MMPs-2	312 (310;317) $p_2 = 0,032$ $p_3 = 0,045$	616 (598;627) $p_1 = 0,012$ $p_3 = 0,046$	254 (252;257)
MMPs-7	4,1 (3,8;4,7) $p_2 = 0,042$ $p_3 = 0,045$	10,4 (11,9;12,5) $p_1 = 0,018$ $p_3 = 0,045$	3,8 (3,6;4,0)
MMPs-9	412 (408;418) $p_2 = 0,013$ $p_3 = 0,056$	864 (840;910) $p_1 = 0,036$ $p_3 = 0,046$	396 (394;398)
первые сутки после операции first day after surgery			
TIMP-1	680 (657;684) $p_2 = 0,038$ $p_3 = 0,045$	436,4 (426,1;442,1) $p_1 = 0,032$ $p_3 = 0,026$	789 (771;793)
MMPs-2	315 (312;317) $p_2 = 0,032$ $p_3 = 0,045$	741 (738;743) $p_1 = 0,012$ $p_3 = 0,045$	254 (252;257)
MMPs-7	4,3 (4,1;4,5) $p_2 = 0,042$ $p_3 = 0,045$	12,3 (11,9;12,5) $p_1 = 0,018$ $p_3 = 0,045$	3,8 (3,6;4,0)
MMPs-9	414 (412;416) $p_2 = 0,013$ $p_3 = 0,056$	952 (950;952) $p_1 = 0,036$ $p_3 = 0,046$	396 (394;398)
третьи сутки после операции third day after surgery			
TIMP-1	787 (776;789) $p_2 = 0,038$ $p_3 = 0,655$	442,4 (440,1;443,6) $p_1 = 0,017$ $p_3 = 0,021$	789 (771;793)
MMPs-2	299 (296;301) $p_2 = 0,023$ $p_3 = 0,021$	741 (738;742) $p_1 = 0,041$ $p_3 = 0,012$	254 (252;257)
MMPs-7	4,2 (3,9;4,5) $p_2 = 0,015$ $p_3 = 0,022$	12,3 (11,2;13,4) $p_1 = 0,038$ $p_3 = 0,026$	3,8 (3,6;4,0)

Исследуемые показатели Researched indicators	Уровень TIMP-1 и MMPs, нг/мл Level of TIMP-1 and MMPs, ng/ml		
	группа 1 ФДТ + операция group 1 PDT + surgery (n=74)	группа 2 операция group 2 surgery (n=111)	группа 3 доноры group 3 donors (n=17)
MMPs-9	401 (398;403) $p_2 = 0,038$ $p_3 = 0,038$	952 (950;953) $p_1 = 0,013$ $p_3 = 0,057$	396 (394;398)
пятые сутки после операции fifth day after surgery			
TIMP-1	783 (782;7840) $p_2 = 0,023$ $p_3 = 0,017$	441,3 (440,2;442,3) $p_1 = 0,022$ $p_3 = 0,017$	789 (771;793)
MMPs-2	249 (247;251) $p_2 = 0,041$ $p_3 = 0,634$	741 (739;742) $p_1 = 0,042$ $p_3 = 0,023$	254 (252;257)
MMPs-7	3,6 (3,4;3,8) $p_2 = 0,021$ $p_3 = 0,634$	12,3 (11,2;13,5) $p_1 = 0,022$ $p_3 = 0,011$	3,8 (3,6;4,0)
MMPs-9	391 (389;393) $p_2 = 0,023$ $p_3 = 0,765$	952 (950;954) $p_1 = 0,017$ $p_3 = 0,028$	396 (394;398)
десятые сутки после операции tenth day after surgery			
TIMP-1	785 (784;787) $p_2 = 0,038$ $p_3 = 0,634$	345,1 (344,7;446,3) $p_1 = 0,048$ $p_3 = 0,012$	789 (771;793)
MMPs-2	248 (247;250) $p_2 = 0,041$ $p_3 = 0,715$	612 (610;614) $p_1 = 0,034$ $p_3 = 0,033$	254 (252;257)
MMPs-7	3,4 (3,1;3,6) $p_2 = 0,021$ $p_3 = 0,715$	10,2 (9,7;11,1) $p_1 = 0,021$ $p_3 = 0,022$	3,8(3,6;4,0)
MMPs-9	395 (393;397) $p_2 = 0,031$ $p_3 = 0,755$	862 (860;864) $p_1 = 0,043$ $p_3 = 0,022$	396 (394;398)

Примечание: данные представлены в виде медианы и межквартильного размаха. Достоверности p1, p2, p3 приведены в соответствии с межгрупповыми сравнениями.
Note: Here and below: data are presented as median and interquartile range. Significances p1, p2, p3 are given in accordance with intergroup comparisons.

In patients included in group 1, the levels of TIMP-1, MMPs-2, MMPs-7 and MMPs-9 did not change compared with the values obtained after PDT.

By the third day after surgery, in patients treated only by the surgical method, there were no statistically significant changes in the level of TIMP-1 and MMPs. Patients who received combined treatment showed a statistically significant increase in the amount of TIMP-1 to the level observed in donors. In these patients, a decrease in the concentration of all studied MMPs was noted, however, compared with the data of donors, their values were statistically significantly higher.

By the fifth day after surgical treatment, there were no statistically significant changes in laboratory parameters in patients of group 2. In patients of group 1 treated with the combined method, it was noted that the values of MMPs-2, MMPs-7 and MMPs-9 decreased statistically significantly and did not differ from the values recorded in the group of healthy donors.

There were no significant changes in the studied parameters in patients with SCSC in both observation groups on the seventh postoperative day; they did not differ statistically significantly from the data observed on the fifth day after surgical treatment.

By the tenth day after the operation, no statistically significant changes were obtained in patients who underwent neoadjuvant PDT. In group 2, there was an increase in the TIMP-1 index and, as a result, a decrease in the levels of the studied MMPs. However, the concentrations of MMPs remained statistically significantly higher than those of donors, their values began to correspond to the results established before the start of surgical treatment.

In the late postoperative period, there were no changes in the analyzed parameters in patients of both groups, all the studied parameters corresponded to the data obtained on the tenth day after the operation.

Thus, as a result of the study, it was found that surgical removal of the tumor without neoadjuvant PDT does not restore the studied parameters. They remained statistically significantly elevated both in the immediate and late postoperative periods. However, the implementation of combined treatment made it possible to increase the level of TIMP-1 statistically significantly before the start of the surgical stage, which naturally led to a decrease in the number of MMPs-2, MMPs-7 and MMPs-9. During dynamic observation, a complete recovery of the TIMP-1 level was determined, which, in turn, contributed to an increase in the number of all MMPs.

During the study, it was found that the TIMP-1 index in group 1 before the start of the surgical stage of treatment was 679 ng/ml, by the 10th day of the postoperative period – 785 ng/ml, in group 2 – 567.3 ng/ml and 345 .1 ng/ml, respectively. The evaluation of the results of the study was carried out by the method of non-parametric statistical analysis in order to establish the reliability of differences in the studied groups using the Mann-Whitney and Kolmogorov-Smirnov criteria. Mann-Whitney test: U = 251.500; Z = 2.927316 ($p = 0.003419$); Kolmogorov-Smirnov criterion: Max Neg Difference = 0.00; Max Pos Difference = 0.433333 ($p < 0.01$).

The initial level of MMPs-2 in group 1 was 312 ng/ml, by day 10 of the postoperative period it was 248 ng/ml; in group 2 – 616 ng/ml and 612 ng/ml, respectively. Mann-Whitney test: U = 117.0000; Z = 4.915821 ($p = 0.000001$); Kolmogorov-Smirnov criterion: Max Neg Difference = 0.00; Max Pos Difference = 0.60000 ($p < 0.001$). The level of MMPs-7 in group 1 was 4.1 ng/ml and 3.4 ng/ml; in group 2 – 10.4 ng/ml and 10.2 ng/ml, respectively. Mann-Whitney test: U = 125.0000; Z = 4.797546 ($p = 0.000002$); Kolmogorov-Smirnov criterion: Max Neg Difference = 0.00; Max Pos Difference = 0.60000 ($p < 0.001$). The level of MMPs-9 in group 1 was 412 ng/ml and 395 ng/ml; in group 2 – 864 ng/ml and 862 ng/ml, respectively. Mann-Whitney test: U = 251.5000; Z=2.927316 (p=0.000001); Kolmogorov-Smirnov criterion: Max Neg Difference = 0.00; Max Pos Difference = 0.60000 ($p < 0.001$). There is a strong feedback ($r = – 0.87$) between the TIMP-1 and MMPs-2, MMPs-7 and MMPs-9 in the blood.

One of the most important criteria for evaluating the effectiveness of cancer treatment is survival, absence of recurrence and disease progression. These indicators were studied in patients included in the 1st and 2nd observation groups. Considering the fact that SCSC was diagnosed at the T1-2N0M0 stage in the patients included in our study, no lethal cases from the underlying disease were recorded during the period of dynamic observation. Fatal outcomes were noted in 12 (6.5%) patients from comorbidities, mainly from cardiovascular events. We consider it necessary to note that all the deceased patients were from group 2, in which only surgical treatment was performed without the use of PDT.

When analyzing other indicators, it was found that metastasis was detected in 19 (10.3%) patients with SCSC in terms of 3 to 7 years. In all cases, metastases in regional lymph nodes were diagnosed, which required chemotherapy. Lymphogenic metastases were predominantly detected in 16 (8.6%) patients of group 2. In the complex treatment group, metastases were found only in 3 (1.6%) patients ($p < 0.05$). It is obvious that the use of the PDT method, and then the implementation of a surgical intervention in the treatment of patients with early stage SCSC, can reduce the risk of lymphogenous metastasis in the long-term period. Relapse of the disease was observed in 15 (8.1%) patients: in group 1 – in 4 (2.2%), in group 2 – in 11 (9.5%) ($p<0.05$). Thus, combined treatment, including PDT before the surgical stage, is an effective method for the treatment of patients with localized SCSC and can be recommended for widespread use.

Discussion

It has been established that changes in TIMP-1, MMPs-2, MMPs-7 and MMPs-9 are recorded already at the early stages of SCSC. Surgical treatment did not lead to the restoration of the initial level of indicators, they remained statistically significantly increased both in the immediate and late postoperative periods. It is possible that SCSC produces substances that reduce the amount of TIMP-1, which, in turn, leads to an increase in the amount of MMPs-2, MMPs-7 and MMPs-9 in the blood. It is known that tissue collagenases MMPs-2 and MMPs-9 hydrolyze type IV collagen, the basis of the basal lamina at the dermoepidermal junction, promoting tumor invasion. In addition, the destruction of type IV collagen contributes to deep damage to epithelial cells from membrane destruction to vascular invasion. MMPs-2 and MMPs-9 also release a number of angiogenic factors, including VEGF, which is considered the main polyclonal inducer of angiogenesis. The destruction of vascular collagen contributes to the disruption of the vascular wall, which leads to endothelial dysfunction [14]. Studies by

foreign scientists [15] have convincingly shown that carcinogenesis is accompanied by imbalance of oncogenes and prooncogenes. These processes lead to hypoxia, induction of angiogenesis (creation of a tumor vascular bed), hydrolysis of the basement membrane and extracellular matrix due to the ability of metalloproteinases to decompose almost all of their components (all types of collagens, elastin, proteoglycans, laminin, and others); tumor progression and metastasis.

The results of our study show that the use of PDT before surgical treatment in patients with early stage SCSC contributes to the normalization of the level of TIMP-1, which leads to the normalization of the level of MMPs-2, MMPs-7 and MMPs-9 in the blood. This prevents damage to the endothelium of the vascular wall both in the immediate and long-term period after treatment [16]. It should be noted that the removal of the tumor without the use of PDT did not lead to correction of the level of metalloproteinases: both before and after surgical treatment, an increase in these indicators was noted. It is possible that surgical treatment without the use of PDT does not lead to the elimination of the causes that induce the development of SCSC. However, this issue requires further research.

Conclusion

The study shows that in patients with the initial stage of SCSC, changes in the parameters of metalloproteinases in the blood serum are recorded, which is manifested by a decrease in the level of TIMP-1 and an increase in MMPs-2, MMPs-7 and MMPs-9. These changes are a trigger for the destruction of vascular collagen and disruption of the integrity of the vascular wall. PDT contributes to the normalization of the levels of TIMP-1 and MMPs-2, MMPs-7 and MMPs-9 in the blood, indirectly prevents damage to the endothelium of the vascular wall and prevents changes in hemostasis.

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THE RESULTS OF THE INDEX COMPARATIVE EVALUATION OF PHOTODYNAMIC THERAPY AND ULTRAVIOLET IRRADIATION IN THE TREATMENT OF CHRONIC GINGIVITIS

Losev K.V., Kostyakova T.V., Batsula N.V., Verendeeva M.A., Kuzina O.V., Losev A.V.
Chuvash State University named after I.N. Ulyanova, Cheboksary, Russia

Abstract

In this clinical study, the effect of photodynamic therapy and ultraviolet radiation on the effectiveness of the treatment of periodontal diseases was examined according to the results of an index assessment. Clinical examination of 95 patients of both sexes aged from 18 to 32 years revealed chronic generalized catarrhal gingivitis (K05.1). The main index criteria for comparative parameters in all study groups were: simplified OHI-S hygiene index, Mullemann-Cowell bleeding index, PMA index. The indices were measured before and after treatment with subsequent control examinations in 1 month, 3 months, 6 months. The patients were divided into three groups and each group underwent standard periodontal treatment aimed at stopping the inflammatory process and preventing its further development. In the first group, the treatment was supplemented with the use of the FotoSan LED lamp with a wavelength of 630 nm, in the second the "Quasar" ultraviolet irradiator was used, in the third (control) group, the complex of therapeutic measures was carried out without physiotherapeutic procedures. According to the results of the study, the use of photodynamic therapy significantly accelerates the regenerative processes of periodontal epithelial tissue and reduces the number of treatment sessions.

Keywords: photodynamic therapy, ultraviolet irradiation, bleeding, gingivitis, hygiene index.

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Contacts: Kostyakova T.V., e-mail: kostyakova-tatyana@list.ru

РЕЗУЛЬТАТЫ ИНДЕКСНОЙ СРАВНИТЕЛЬНОЙ ОЦЕНКИ ПРИМЕНЕНИЯ ФОТОДИНАМИЧЕСКОЙ ТЕРАПИИ И УЛЬТРАФИОЛЕТОВОГО ОБЛУЧЕНИЯ ПРИ ЛЕЧЕНИИ ХРОНИЧЕСКОГО ГИНГИВИТА

К.В. Лосев, Т.В. Костякова, Н.В. Бацула, М.А. Верендеева, О.В. Кузина, А.В. Лосев
Чувашский государственный университет им. И.Н. Ульянова, Чебоксары, Россия

Резюме

В клиническом исследовании рассмотрено влияние фотодинамической терапии и ультрафиолетового облучения на эффективность лечения заболеваний пародонта по результатам индексной оценки. В результате клинического осмотра у 95 пациентов обоих полов в возрасте от 18 до 32 лет был выявлен хронический генерализованный катаральный гингивит (К05.1). Основными индексными критериями сравнительных параметров во всех группах исследования являлись: упрощенный индекс гигиены по OHI-S, индекс кровоточивости по Мюллеману-Коуэллу, индекс PMA. Показатели измерялись до и после лечения с последующими контрольными осмотрами через 1 мес, 3 мес, 6 мес. Пациенты были разделены на три группы, в каждой группе проводилось стандартное пародонтологическое лечение, направленное на купирование воспалительного процесса и предупреждение дальнейшего его развития. В первой группе лечение дополнялось использованием светодиодной лампы FotoSan с длиной волны 630 нм, во второй группе применялся ультрафиолетовый облучатель «Квазар», в третьей (контрольной) группе комплекс лечебных мероприятий проводился без физиотерапевтических процедур. По результатам исследования применение фотодинамической терапии значительно ускоряет регенераторные процессы эпителиальной ткани пародонта и сокращает количество сеансов лечения.

Ключевые слова: фотодинамическая терапия, ультрафиолетовое облучение, кровоточивость, гингивит, индекс гигиены.

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Контакты: Костякова Т.В., e-mail: kostyakova-tatyana@list.ru

Introduction

The state of health of organs and tissues of the oral cavity has a great influence on the quality of life and well-being of a person [1].

Periodontal diseases are a serious public health problem affecting more than half of the adult population worldwide [2], and have not only medical but also social significance due to the high incidence, great variety and severity of the course [3]. The presence of chronic infection foci in the human body can cause the development of a large number of diseases and negatively affect the state of the dental system [4].

Gingivitis is an inflammatory process of the gum mucosa caused by the presence of a bacterial biofilm. The main clinical manifestations of gingivitis are gingival redness, bleeding [5] and edema [6].

In the treatment of periodontal diseases, physiotherapeutic methods have found wide application [7].

Photodynamic therapy (PDT) is based on the use of photosensitizers and light of a certain wavelength [8]. Photosensitizers are activated by low-intensity laser radiation with a wavelength corresponding to the absorption peak of the photosensitizer [9]. The photosensitizer toluidine blue can selectively accumulate inside the mitochondria of bacterial cells and causes a significant reduction in the number of cariogenic species. Toluidine blue can easily penetrate the bacterial membrane, has a transmembrane permeability coefficient higher than other photosensitizers, has low toxicity to human cells, a high rate of generation of reactive oxygen species and versatility due to a wide absorption band, which allows it to be activated using many light sources [10]. As a result of the photochemical reaction, negatively charged radicals are released, which have a pronounced bactericidal activity, stimulating tissue proliferation and regeneration [11].

Ultraviolet radiation is a spectrum of electromagnetic vibrations in the range from 180 to 400 nm. In terms of its activity, it significantly exceeds all other parts of the light spectrum, but, given the smallest depth of penetration into tissues – only up to 1 mm, their direct effect is limited to the surface layers of the irradiated areas of the skin and mucous membranes. Ultraviolet radiation increases the activity of protective mechanisms, has a desensitizing effect, normalizes blood coagulation processes, and improves lipid metabolism [12].

The aim of this study was to study the effectiveness of combined treatment of periodontal diseases, including standard therapy with the additional use of photodynamic therapy or ultraviolet irradiation.

Materials and methods

The study involved 95 patients of both sexes diagnosed with chronic generalized catarrhal gingivitis (K05.1). The diagnosis was made on the basis of the clinical picture and panoramic x-ray data and clarified in accordance with the classification of periodontal diseases adopted at a meeting of the presidium of the periodontology section of the Russian Academy of Dentistry in 2001.

The study was approved by the local ethics committee of the Federal State Budgetary Institution of Higher Education I. N. Ulianov Chuvash State University and was carried out in accordance with the principles of the Declaration of Helsinki of the World Association "Ethical principles for conducting scientific medical research involving humans" (as amended by the 64th WMA General Assembly, Fortaleza, Brazil, October 2013).

Criteria for inclusion in the research work were: availability of informed voluntary consent to participate in a clinical trial; age of patients from 18 to 32 years; absence of chronic somatic diseases and aggravated allergic anamnesis; the presence of hyperemia and bleeding gums.

Exclusion criteria: benign and malignant neoplasms; acute and chronic infectious and viral diseases; 2 and 3 degrees hypertension; pregnancy and lactation; somatic diseases in the acute stage; systemic blood diseases; hypersensitivity to ultraviolet radiation and intolerance to the components of the photosensitizer.

In the study groups, an objective examination revealed the presence of a burdened local dental status: dental plaque (biofilm) and mineralized dental deposits.

The complex of periodontal treatment in all groups consisted of the following stages: professional hygiene followed by coating of the teeth with a fluorine-containing agent, the appointment of local anti-inflammatory and antiseptic therapy.

All patients were informed about the need to comply with the terms of attendance at all stages of treatment. During the general clinical examination, the anamnesis, complaints (bleeding, pain in inflamed gums, hyperemia of the gingival margin) and objective data were taken into account. During the study, the main criteria for comparative parameters in all study groups before and after the start of treatment were: hygiene index (HI) according to OHI-S (Green, Vermillion, 1964), bleeding index according to Muhlemann-Cowell (Muhlemann-Cowell; 1975), PMA index (C. Parma, 1960). The terms of control visits with the measurement of indicators were 1 month, 3 months, 6 months.

The patients were divided into three groups. The first group consisted of 32 (33.7%) patients, the second – 31 (32.6%), the third (control group) – 32 (33.7%) patients. Participants of all groups underwent professional oral hygiene with controlled brushing. Patients were taught the basic rules of oral care, and they were also given a selection of items and hygiene products. At home, the study participants used toothpaste based on herbal extracts (sage, echinacea, myrrh, chamomile, ratanya and mint) (GlascoSmithKline Healthcare JSC, Russia), sodium bicarbonate and sodium fluoride (1400

ppm), alcohol-free rinse based on an aqueous solution of chlorhexidine digluconate (0.2%) and sodium fluoride (250 ppm).

To conduct PDT in the first group, the photosensitizer toluidine blue was used at a concentration of 1 mg/ml. Irradiation was carried out using a FotoSan 630 LED lamp with a wavelength of 620-640 nm for 10 seconds on the area of each tooth with an inflamed gum area. The treatment included 3 courses of PDT, the interval between courses was 4 days.

In the second group, ultraviolet radiation was used with the Kvazar device through a tube in the spectral range from 205 to 315 nm, each quadrant of the upper and lower jaws was irradiated in turn. Duration of exposure: 1/2 biodoses for each area with a gradual increase to 2 biodoses. The treatment course included 10 procedures.

The treatment tactics of participants in the third control group did not include physiotherapeutic methods of influence.

All participants were taken for dispensary registration with the registration of periodontal parameters.

When analyzing statistical indicators, the program "Statistica 6.0" was used. In evaluating the results, the data of the mean and its standard deviation (M±m) were taken into account. The parameters compared in the groups were evaluated using the Mann-Whitney U-test. At $p<0.05$, the differences were considered statistically significant.

Результаты

In the course of a clinical study, during the initial examination, the presence of soft and hard dental deposits was determined in all 95 patients, hyperemia and swelling in the area of the gingival papillae and marginal gums were found in 78 (82.1%), signs of congestive hyperemia (cyanotic coloration) – in 17 (26.15%), gum bleeding – in 82 (86.31%) patients.

Signs of destruction of the interalveolar septa on orthopantomographies in patients of all observation groups were not detected.

Prior to the start of treatment, in all groups, IG indicators were at the same level and corresponded to indicators of poor hygiene (see Table 1). After professional hygiene, teaching the rules of brushing teeth and individual selection of items and hygiene products, the index score improved in most of the subjects throughout the course of treatment.

At the initial examination (see Table 2), the data of the periodontal PMA index in all groups corresponded to the average degree of inflammation. The indicators after 1 and 3 months in all experimental groups were minimal, and the differences between them were not statistically significant. After 6 months, in the second and control groups, an increase in the index was noted compared to the first group, that is, in patients treated with PDT, a more stable remission was obtained.

The bleeding index (see Table 3) at the initial visual examination in all compared groups indicated an average degree of inflammation, which indicates bleeding of the gingival sulcus and gingival hyperemia. Evaluation of the bleeding index after 1 month showed a decrease in its value in all study groups. After 3 months, the bleeding index values were higher compared to the previous values in all three observation groups. How-

Таблица 1
Динамика показателя индекса гигиены
Table 1
Dynamics of the hygiene index

Обследуемые группы Study groups	Индекс гигиены (M ± m) Hygiene index (M ± SE)			
	До лечения Before treatment	После лечения After treatment		
		1 мес 1 month	3 мес 3 months	6 мес 6 months
1 группа – ФДТ Group I – PDT	2,69±0,08	0,42±0,02*	1,04±0,09**	1,36±0,06**
2 группа – УФО Group II – UVI	2,71±0,08	0,49±0,02*	1,28±0,06**	1,52±0,07
Контрольная группа Control group	2,64±0,07	0,55±0,03	1,42±0,07	1,59±0,08

Примечание: * – статистически значимая достоверная разница по сравнению со значениями до лечения ($p<0,05$); ** – статистически значимая достоверная разница показателей по сравнению с контрольной группой в этот же период наблюдения ($p<0,05$).
Note: * – statistically significant difference compared to the values before treatment ($p<0.05$); ** – statistically significant difference compared to the control group during the same observation period ($p<0.05$).

ever, compared with the control, statistically significant lower index values were recorded in groups in which physiotherapy was additionally used. After 6 months of follow-up, significant differences persisted only in the PDT group.

A dynamic evaluation of the effectiveness of periodontal treatment revealed that in the first group after PDT using the FotoSan LED lamp, 2 (6.25%) patients complained of bleeding after brushing their teeth throughout the entire course of treatment, HI remained unsatisfactory (1.7), the index bleeding corresponded to 0.5, PMA – 27%.

There were complaints of bleeding after brushing the teeth during the entire course of treatment in 6 (19.3%) patients who received Ultraviolet irradiation sessions. HI remained unsatisfactory (1.8), bleeding index was 0.8, PMA – 35%.

In the control group, the persistence of complaints

Таблица 2
Динамика показателя пародонтологического индекса
Table 2
Dynamics of periodontal index

Обследуемые группы Study groups	Индекс PMA (M ± m) PMA index (M ± SE)			
	До лечения Before treatment	После лечения After treatment		
		1 мес 1 month	3 мес 3 months	6 мес 6 months
1 группа – ФДТ Group I – PDT	51,27±2,12	10,69±0,51*	14,54±0,7**	18,54±0,89**
2 группа – УФО Group II – UVI	55,04±2,25	13,12±0,61*	15,97±0,71**	27,73±1,29
Контрольная группа Control group	53,69±2,21	13,81±0,65	17,5±0,81	28,42±1,33

Примечание: * – статистически значимая достоверная разница по сравнению со значениями до лечения (p<0,05); ** – статистически значимая достоверная разница показателей по сравнению с контрольной группой в этот же период наблюдения (p<0,05).
Note: * – statistically significant difference compared to the values before treatment (p<0.05); ** – statistically significant difference compared to the control group during the same observation period (p<0.05).

Таблица 3
Динамика показателя индекса кровоточивости
Table 3
Dynamics of the bleeding index

Обследуемые группы Study groups	Индекс кровоточивости (M ± m) Bleeding index (M ± SE)			
	До лечения Before treatment	После лечения After treatment		
		1 мес 1 month	3 мес 3 months	6 мес 6 months
1 группа – ФДТ Group I – PDT	1,82±0,03	0,16±0,075*	0,30±0,013**	0,37±0,05**
2 группа – УФО Group II – UVI	1,86±0,03	0,25±0,01*	0,45±0,02**	0,60±0,06
Контрольная группа Control group	1,84±0,03	0,28±0,012	0,53±0,026	0,64±0,06

Примечание: * – статистически значимая достоверная разница по сравнению со значениями до лечения (p<0,05); ** – статистически значимая достоверная разница показателей по сравнению с контрольной группой в этот же период наблюдения (p<0,05).
Note: * – statistically significant difference compared to the values before treatment (p<0.05); ** – statistically significant difference compared to the control group during the same observation period (p<0.05).

of bleeding after brushing the teeth during the entire course of treatment was noted in 9 (28.1%) patients. HI remained unsatisfactory (1.8), bleeding index was 1.2, PMA – 40%.

Dispensary follow-up of the study participants for 12 months revealed a more stable remission and improved diagnostic criteria in patients who received additional PDT treatment (see Fig.). The index of HI in the group became satisfactory, but changed insignificantly.

In the second and control groups, an increase in HI was observed, which indicated an unsatisfactory result of treatment. The bleeding index showed a mild degree of inflammation in all groups. The values of the PMA index in patients of the second and control groups corresponded to the average degree of inflammation, in the group with the use of PDT, a slight increase in the index was noted.

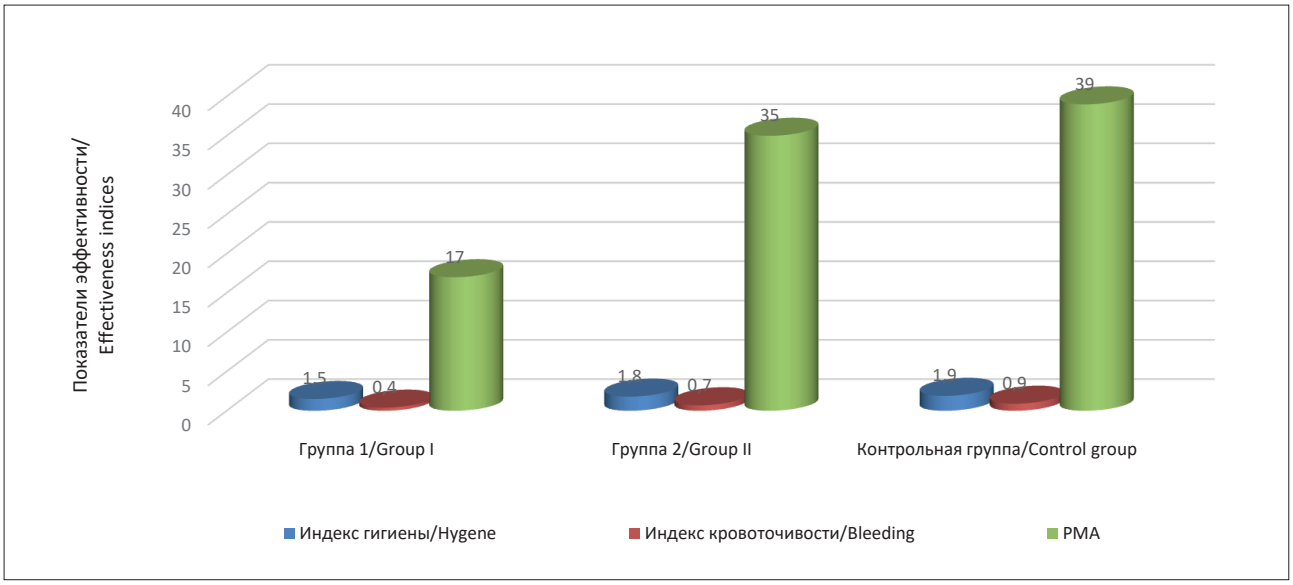

Рис. Показатели индексной сравнительной оценки эффективности лечения хронического гингивита через 12 мес.
Fig. Comparative assessment of the effectiveness indices of chronic gingivitis treatment after 12 months.

Discussion

The results of this study highlight the importance of developing preventive and therapeutic measures aimed at preventing the progression of periodontal disease.

In particular, chronic generalized catarrhal gingivitis can lead to periodontitis, which causes resorption of the alveolar bone and subsequent loss of teeth, and can exacerbate the risk of developing various systemic diseases such as diabetes, rheumatoid arthritis, and inflammatory diseases of the gastrointestinal tract [13].

During the examination of the oral cavity, the dentist can obtain information about the severity of the disease using index indicators [14]. Clinical indices used in the research part of the work assess the condition of the periodontium, objectifying information about the clinical picture in the mouth and the level of hygiene care.

According to the primary examination in patients of all groups, the presence of soft and hard dental deposits was determined. To improve the hygienic condition and reduce the periodontopathogenic microflora, professional controlled hygiene and the selection of oral cavity care items and products were carried out, as a result of which the clinical indicators of the periodontium improved.

In the practice of providing dental care, the importance of restorative and rehabilitation technologies has increased significantly, among which physical methods play a leading role. Physical factors, having an immunocorrective and healing effect, affect the body as a whole, change the physicochemical properties of cells and the metabolic processes occurring in them at the cellular level [15].

The results of the study showed that patients who, in addition to the main one, received physiotherapeutic treatment in the form of PDT or ultraviolet irradiation, showed better dynamics of recovery compared to the control group. Comparison of the two physiotherapeutic methods showed significantly better index values for PDT local impact on inflammation foci both after the end of therapeutic measures and during follow-up monitoring.

Currently, many researchers are turning to studying the possibilities of PDT, which is widely used in various fields of medicine [16, 17].

Conclusion

According to the results of the index evaluation in the treatment of chronic generalized catarrhal gingivitis, the therapeutic effect of PDT was more effective than ultraviolet irradiation.

The use of PDT is an effective non-invasive additional treatment for patients with periodontal diseases, it contributes to a more rapid decrease in the inflammatory process and prolongs the period of disease remission.

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INFLUENCE OF ROSE BENGAL ON PLATELET AGGREGATION ACTIVITY

Petrishchev N.N., Grishacheva T.G., Chefu S.G.
Pavlov First Saint-Petersburg State Medical University, Saint-Petersburg, Russia

Abstract

The goal of the study is to comparatively examine the effect of photoactivated rose bengal on platelet aggregation *in vitro* and in circulating blood of male Wistar rats. Platelet-rich plasma (PRP) was obtained from venous blood. The aggregation activity of platelets was determined by the turbidimetric method, the aggregation inducer was ADP at a final concentration of 1.25 μM. Rose bengal (RB) (Acros Organics, USA) was used as a photosensitizer (PS). PRP samples containing the PS were irradiated using ALOD-Izumrud laser (OOO "Alcom Medica", Russia), λ = 532 nm, power density 0.05 W/cm^2, energy density of 6, 12 and 24 J/cm^2. The effect of photoactivated RB on the aggregation of circulating PLT was studied after laser irradiation of the femoral artery of the rats: 30 mW laser power, 2 mm spot diameter and 30 min exposure. RB at concentrations of 0.5 and 1 μg/ml was found to stimulate, and 5-10 μg/ml—to inhibit platelet aggregation. Photoactivation of RB weakens the stimulating effect of laser irradiation on the aggregation of platelets. Photodynamic modification of blood led to an increase in the intensity of platelet aggregation by 24% in comparison to the control group, and by 39.6% compared to the group without photoactivation of RB ($p<0.01$). The data obtained indicate that under the influence of RB photoactivation, the aggregation activity of platelets changes, the severity and direction of the effect depend on the RB concentration. Change in functional activity of platelets is one of the manifestations of photodynamic modification of blood.

Keywords: rose bengal, photoactivation, photodynamic blood modification, platelet rich plasma, platelet aggregation.

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Contacts: Grishacheva T.G., e-mail: laser82@mail.ru

ВЛИЯНИЕ БЕНГАЛЬСКОГО РОЗОВОГО НА АГРЕГАЦИОННУЮ АКТИВНОСТЬ ТРОМБОЦИТОВ

Н.Н. Петрищев, Т.Г. Гришачева, С.Г. Чефу
Первый Санкт-Петербургский государственный медицинский университет им. акад. И.П. Павлова, Санкт-Петербург, Россия

Резюме

Проведено сравнительное изучение влияния фотоактивированного бенгальского розового на агрегацию тромбоцитов *in vitro* и в циркулирующей крови крыс-самцов Wistar. Из венозной крови получали плазму, обогащенную тромбоцитами (PRP). Агрегационную активность тромбоцитов определяли турбодиметрическим методом, индуктор агрегации – АДФ в конечной концентрации 1,25 μM. В качестве фотосенсибилизатора (ФС) использовали бенгальский розовый (БР) (Acros Organics, США). Пробы PRP, содержащие ФС, облучали с помощью лазерного аппарата АЛОД-Изумруд (ООО «Алком медика», Россия), λ=532 нм, плотность мощности 0,05 Вт/см^2, плотность энергии 6, 12, 24 Дж/см^2. Влияние фотоактивированного БР на агрегацию циркулирующих тромбоцитов изучали после лазерного облучения бедренной артерии крыс. Параметры облучения: мощность 30 мВт; диаметр пятна 2 мм; экспозиция 30 мин. БР в концентрациях 0,5 и 1 мкг/мл стимулирует, а 5–10 мкг/мл – угнетает агрегацию тромбоцитов. Фотоактивация БР ослабляет стимулирующее действие лазерного облучения на агрегацию тромбоцитов. Фотодинамическая модификация крови приводила к увеличению интенсивности агрегации тромбоцитов на 24% по сравнению с контрольной группой, на 39,6% – по сравнению с группой без фотоактивации БР ($p<0,01$). Полученные данные свидетельствуют о том, что под влиянием фотоактивации БР изменяется агрегационная активность тромбоцитов, степень выраженности и направленность эффекта зависят от концентрации БР. Изменение функциональной активности тромбоцитов является одним из проявлений фотодинамической модификации крови.

Ключевые слова: бенгальский розовый, фотоактивация, фотодинамическая модификация крови, плазма богатая тромбоцитами, агрегация тромбоцитов.

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Контакты: Гришачева Т.Г., e-mail: laser82@mail.ru

Introduction

The presence of a halogenated xanthene ring in the structure of rose bengal (RB) determines its properties as a photosensitizer (PS). When irradiated with green light (maximum absorption at a wavelength of 546 nm), the RB goes into an excited state and photochemical reactions develop.

RB as a PS is used in oncology, ophthalmology, and some other areas of clinical medicine, as well as in experimental studies [1, 2]. In 1985, B.D. Watson et al. showed for the first time that the irradiation of vessels with green light against the background of preliminary administration of RB naturally leads to the formation of a thrombus [3]. In subsequent studies, it was found that RB is taken up by the endothelium and activated upon subsequent irradiation. In this case, reactive oxygen species are formed, including singlet oxygen, causing photodynamic damage to the endothelium, which manifests itself in the release of thrombogenic substances from it, the expression of adhesion molecules, which initiates the formation of a thrombus [4-6].

RB circulating in the blood is absorbed not only by the endothelium, but also by other cells, including blood cells. During PDT of tumors, photoactivation of RB fixed in the endothelium and, possibly, in blood cells circulating in the irradiation zone occurs. The question of the role of platelets that have experienced photodynamic effects in the formation of a thrombus remains open. According to J. Inamo et al. (1996) photoactivated RB has a weak activating effect on ADP-induced human thrombocyte aggregation (*in vitro*). Based on these data, the authors concluded that RB photoactivation is not of great importance in the development of photodynamically induced thrombosis [7].

The aim of our study was a comparative study of the effect of photoactivated RB on platelet aggregation *in vitro* and in circulating blood.

Materials and methods

The experiments were performed on male Wistar rats (FSUP "Rappolovo" Nursery of Laboratory Animals", FSBI "National Research Center "Kurchatov Institute"). The animals were kept and cared for in accordance with the rules set forth in the European Convention for the Protection of Vertebrate Animals (Strasbourg, 1986). The study was approved by the commission for the maintenance and use of vertebrate laboratory animals at The Pavlov First Saint Petersburg State Medical University as part of the State R&D assignment No. AAAA-A18-118091790075-0 "Development of the principles of laser photodynamic theranostics" (2015–2020).

Platelet aggregation was studied by the turbodimetric method using an AT-02 aggregometer (Russia).

Blood was taken from the jugular vein of anesthetized rats (20% urethane solution, 5 ml/kg body weight, intraperitoneally). A 3.2% sodium citrate solution in a ratio of 9:1 was used as a blood stabilizer. Blood centrifugation mode: to obtain platelet-rich plasma (PRP) – 200 g, 10 min; platelet-poor plasma (PPP) – 1700 g, 30 min.

Platelet aggregation inducer – ADP (Chrono-log Co, USA) at a final concentration of 1.25 μM. The following aggregation parameters were determined: aggregation intensity – increase in light transmission (MA) %; time to reach MA, (t_1) s; aggregation rate – V_{agr} (MA/t_1); MA reduction time by 2 times (t_2) s; disaggregation rate – V_{desagr} ($½\ MA/t_2$).

In experiments *in vitro*, RB (Acros organics, USA) was added to plasma containing a standard number of platelets ($270–350*10^9$/l) at concentrations from 0.5 to 10 μg/ml in experiments to study the effect of RB on platelet aggregation activity without photoactivation.

In experiments to study the effect of photoactivated RB on platelet aggregation *in vitro*, RB was added at a certain concentration of 5 μg/ml to plasma containing a standard number of platelets. After a 5-minute incubation in the dark, the sample was irradiated and platelet aggregation activity was determined. In the comparison groups, the effect of RB (without irradiation) and laser exposure (without RB) on platelet aggregation was studied.

Irradiation procedure

Samples of PRP with a volume of 370 μl were poured into the cells of a 24-well plate and irradiated in the dark using a semiconductor laser device (ALOD, Russia). The end of the light guide was fixed on a tripod and placed at a distance of 10 mm from the plate surface (see Fig. 1). Irradiation parameters: wavelength 532 nm, laser power 0.5 W, power density 0.05 W/cm^2, energy density 6 J/cm^2 (2 min exposure), energy density 12 J/cm^2 (4 min exposure), energy density 24 J/cm^2 (8 min exposure). The laser radiation power was controlled using a power meter (Advantest Q8230, USA) before each experiment.

The study of the effect of photoactivated RB on circulating platelets was carried out as follows: 1 hour after intravenous administration of RB under conditions of anesthesia in rats, a section of the femoral artery was isolated from the neurovascular bundle (see Fig. 2) and supravascular laser irradiation was performed using a focuser (Alcom Medica LLC, Russia). Irradiation conditions: λ = 532 nm, power 30 mW, spot diameter 2 mm, power density 0.9 W/cm^2, exposure 30 min, energy density 1620 J/cm^2. The parameters of laser radiation were chosen according to the data obtained in previous studies, in which RB photoactivation led to a guaranteed decrease in the blood flow velocity in the vessels and the formation of a thrombus [4]. Irradiation in the same modes of the femoral artery in rats without prior administration of RB did not lead to the formation of a thrombus.

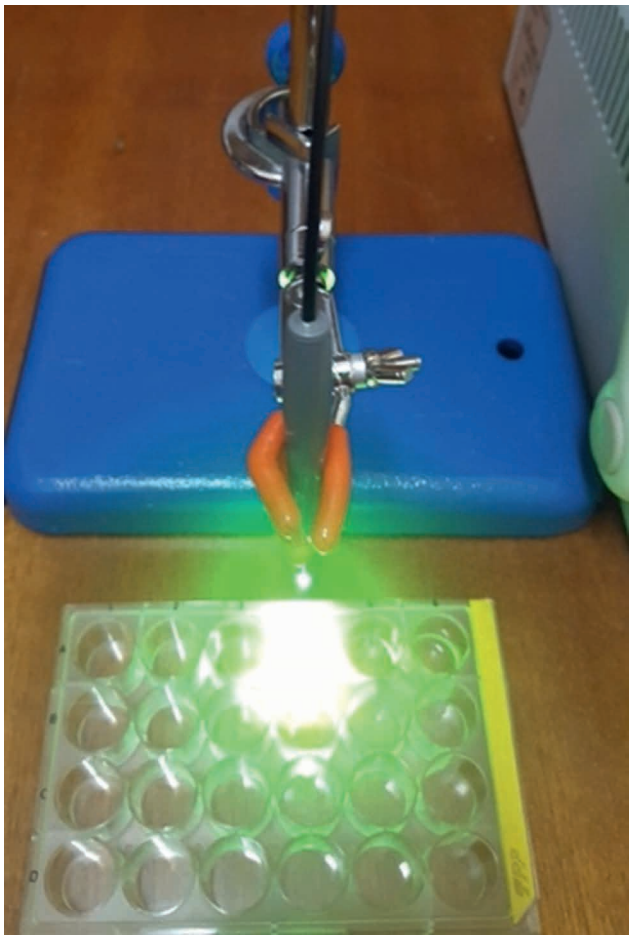

Рис. 1. Процедура облучения *in vitro* PRP в 24-луночном планшете.
Fig. 1. *In vitro* irradiation procedure of platelet-rich plasma in 24 well plate.

After completion of irradiation, blood was taken and platelet aggregation was examined. In the comparison groups, the effect of supravascular irradiation of the femoral artery (without RB) and intravenous administration of RB (without irradiation) on platelet aggregation was evaluated.

Statistical data processing

Data collection was carried out using a spreadsheet Microsoft Excel 2007. Quantitative data were tested for normal distribution using the Shapiro-Wilk W test. We used non-parametric methods of statistical analysis using the Mann-Whitney test. Numerical data are presented as median (lower quartile/upper quartile). The significance of the established differences was judged by the level of values $p<0.05$.

Results

In experiments *in vitro*, RB was added to PRP (final concentration from 0.5 to 10 μg/ml) and platelet aggregation was examined after a 5-minute incubation in the dark. As can be seen from the data in Fig. 3, at a concentration of 0.5 and 1 μg/ml, MA increased by 52.3 and 34.6%, respectively, compared with the control ($p<0.01$), while the rate of aggregation and disaggregation did not change significantly. The intensity of platelet aggregation at a RB concentration of 2.5 μg/ml did not differ from the control, however, the rate of aggregation and disaggregation slowed down significantly by 22.2 and 26%, respectively. An increase in the concentration of RB to 5 and 10 μg/ml led to a decrease in the intensity of platelet aggregation by 43.9 and 53.3%, respectively. The rates of aggregation and disaggregation decreased significantly ($p<0.01$).

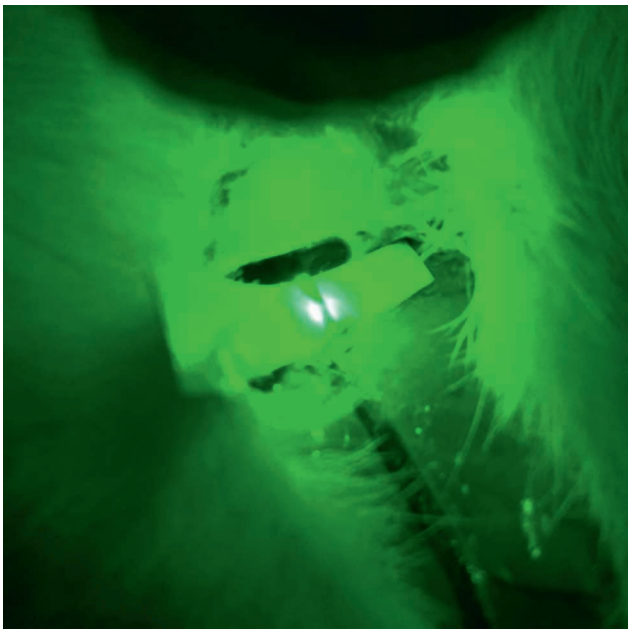

Рис. 2. Надсосудистое лазерное облучение бедренной артерии у крыс.
Fig. 2. Supravascular laser irradiation of the femoral artery in rats.

Thus, the direction and severity of the effect of RB on platelet aggregation *in vitro* depended on the concentration: at low concentrations (0.5 and 1 μg/ml), stimulation was observed, and at higher concentrations, inhibition.

PRP irradiation (without RB) significantly increased the intensity of platelet aggregation compared to the control: at 6 J/cm^2, MA increased by 55.1%; at 12 J/cm^2 – by 65.4%; at 24 J/cm^2 – by 90.7% ($p<0.01$). The rates of aggregation and disaggregation did not change significantly.

In the next group of experiments, after a 5-minute incubation of PRP with RB at a concentration of 5 μg/ml, the samples in the dark were subjected to laser irradiation with an energy density of 12 J/cm^2.

As can be seen from Fig. 4, laser irradiation (532 nm) of PRP after incubation with RB (5 μg/ml) led to a decrease in MA, while irradiation without RB increased the intensity of aggregation ($p<0.01$). Thus, RB photoactiva-

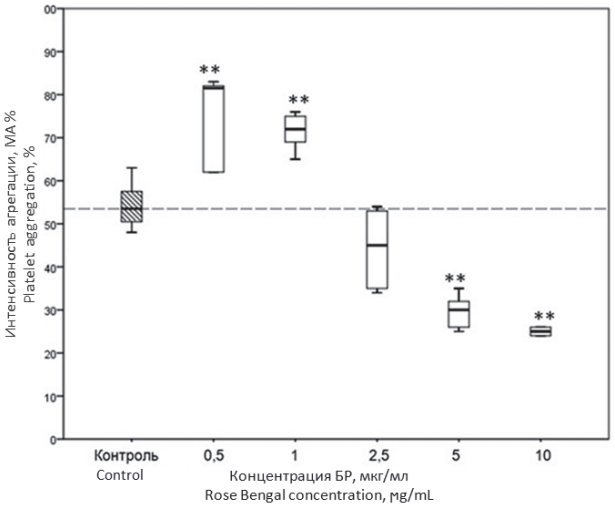

Рис. 3. Влияние бенгальского розового на интенсивность агрегации тромбоцитов.
Примечание: ** – p<0,01 по сравнению с контролем.
Fig. 3. Influence of rose bengal on the intensity of platelet aggregation.
Note:** – p <0.01 compared to control.

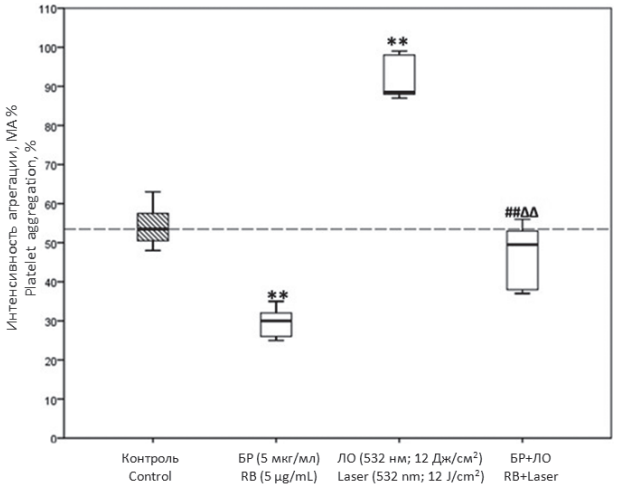

Рис. 4. Влияние БР, лазерного облучения, лазерного облучения на фоне предварительного добавления в PRP БР на интенсивность агрегации тромбоцитов.
Примечание: ** – p<0,01 по сравнению с контролем; ## – p<0,01 по сравнению с БР; ΔΔ – p<0,01 по сравнению с группой облучения.
Fig. 4. Influence of the RB, laser irradiation, laser irradiation with addition of RB to platelet-rich plasma on the intensity of platelet aggregation.
Note: ** – p <0.01 compared to control; ## – p <0.01 compared to RB; ΔΔ – p <0.01 in comparison with the irradiation group.

tion weakens the stimulating effect of laser irradiation on platelet aggregation (Fig. 4).

Photodynamic modification of circulating blood in our studies was carried out by irradiating the femoral artery in rats against the background of preliminary administration of RB.

As can be seen from the table, one hour after the intravenous administration of RB, the intensity of platelet aggregation did not change significantly, however, the time to reach MA was 20.7% less versus control (p<0.01).

Thus, prolonged contact of circulating platelets with RB affected their functional activity, but to a lesser extent than in *in vitro* experiments.

Irradiation of the femoral artery in rats without prior administration of RB (blood photomodification) did not lead to a significant change in platelet aggregation activity.

Photodynamic modification of blood (irradiation of the artery) led to an increase in the intensity of platelet aggregation by 24% compared with the control group, and by 39.6% compared with the group without RB photoactivation (p<0.01). The aggregation rate increased by 36.6% compared to the control group and by 27.3% compared to the group without RB photoactivation (p<0.05) (see Table). The disaggregation rate did not differ from the data in other groups.

Discussion

Weak lipophilicity, the presence of a double negative charge at physiological pH limits the penetration of RB into cells at low concentrations and in the absence of carriers, such as albumin. This explains the fact that in an aqueous solution RB has practically no effect on normal cells, while at the same concentrations it has a cytotoxic effect on the cultured cells of some tumors [1, 2, 8, 9].

In our experiments, it was found that RB has an effect on platelet aggregation, while the degree of severity and direction of the effect depend on the concentration of RB. The penetration of RB into intact platelets most likely occurs by endocytosis of the RB complex with albumin. There is evidence in the literature that some PS have a direct effect on platelets, but most authors observed an inhibitory effect only after PS photoactivation [10-13].

According to J. Inamo et al. RB at a final concentration of 5 μg/ml did not affect ADP-induced aggregation of human platelets. According to our data, RB at concentrations of 5 and 10 μg/ml naturally inhibited rat platelet aggregation, and at lower concentrations (0.5–1.0 μg/ml) stimulated it. Perhaps these differences are associated with the specific features of human platelets. J. Inamo et al. noted some stimulating effect of photoactivated RB on platelet aggregation (532 nm, dose not specified), but this effect did not differ from the effect of irradiation itself on platelets.

Changes in the functional state of circulating platelets during irradiation of the femoral artery of rats against the background of preliminary administration of RB can be the result of both direct and indirect photodynamic effects. In the 90s of the last century, V.H. Fingar et al. showed that during PDT of experi-

Таблица
Влияние фотодинамической модификации крови на показатели АДФ-индуцированной агрегации тромбоцитов
Table
Influence of photodynamic blood modification on indicators of ADP-induced platelet aggregation

Группа Group	Показатели агрегации Aggregation parameters				
	Максимальная амплитуда агрегации (МА), % Maximum aggregation amplitude (MA), %	Время достижения МА, с Time to reach MA, s	Время уменьшения МА в 2 раза, с Time to decrease MA by 2 times, s	Скорость агрегации, %/с Aggregation rate, %/s	Скорость дезагрегации, %/с Disaggregation rate, %/s
Контроль Control (n=15)	54 (52–58)	130 (113–143)	219 (192–251)	0,41 (0,38–0,48)	0,31 (0,25–0,35)
БР (17 мг/кг) RB (17 mg/kg) (n=6)	48 (33–54)	103 (97–112)**	173 (153–186)**	0,44 (0,34–0,47)	0,32 (0,31–0,32)
ЛО 532 нм LI 532 nm (n=6)	57,5 (52–60)	126,5 (122–141)	222,5 (202–249)	0,45 (0,42–0,47)	0,31 (0,3–0,32)
БР+ЛО RB+LI (n=8)	67 (61–77)**## Δ	126 (103–146)	226 (179–264)	0,56 (0,48–0,61)* # Δ	0,37 (0,31–0,44)

Примечание: n — число животных; * – p<0,05 по сравнению с контролем; ** – p<0,01 по сравнению с контролем; # – p<0,05 по сравнению с группой ФС; ## – p<0,01 по сравнению с группой ФС; Δ – p<0,05 по сравнению с группой облучения. БР – бенгальский розовый; ЛО – лазерное облучение; РБ+ЛО – лазерное облучение после предварительного введения БР.
Note: n — number of animals; * – p<0.05 compared to control; ** – p<0.01 compared to control; # – p<0.05 compared to the RB group; ## – p<0.01 compared to the RB group; Δ – p<0.05 compared to the irradiation group. RB - rose bengal; LI – laser irradiation; RB+LI - laser irradiation after preliminary administration of RB.

mental tumors, the content of thromboxane A2 (TxA2) in the blood increases. The authors explain this by the release of TxA2 from the endothelium of tumor vessels in the zone of photodynamic exposure [14]. TxA2 is known to be a platelet activator, which, according to the authors, promotes thrombus formation in tumor vessels during PDT. The described mechanism of changes in platelet activity during PDT can be considered as indirect, that is, not associated with direct photodynamic damage to circulating platelets. In our experiments, the femoral artery was irradiated against the background of preliminary RB injection. In previous studies, it was shown that with the experimental design used, photodynamically induced thrombi are formed in all cases, that is, there was damage to the endothelium in the irradiation zone [4, 18]. However, the damage area (3.14 mm^2) was incommensurably smaller than in the experiments of V.H. Fingar et al. during irradiation of tumor vessels.

There is evidence in the literature that intravenous and supravascular laser irradiation of blood after the preliminary administration of PS has a cytotoxic effect on circulating tumor cells. This effect is considered as a result of photodynamic modification of blood [15-18]. It can be assumed that platelets circulating in the blood in the area of femoral artery irradiation also develop photochemical processes that affect their functional state, that is, there is a direct photodynamic effect.

Conclusion

In our study, RB, like some other PS in our previous experiments, had a direct effect on platelets and their functional activity *in vitro* and *in vivo* [19]. The severity of the effect *in vitro* depends on the concentration of the drug. At low concentrations of RB, stimulation of the aggregation activity of platelets is observed, at high concentrations – inhibition. Unlike PS of the chlorine series, photoactivation of RB at a concentration of 5 μg/ml did not increase the inhibitory effect of platelet aggregation under *in vitro* conditions [19].

It has been shown for the first time that photomodification of blood against the background of preliminary administration of RB leads to moderate activation of the functional activity of platelets. A change in the functional activity of platelets is one of the manifestations of photodynamic modification of blood. Considering that the photodynamic model of thrombosis using RB is widely used in preclinical studies, these data should be taken into account when studying the effectiveness of antithrombotic agents.

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PHOTODYNAMIC THERAPY IN THE TREATMENT OF PATIENTS WITH MYCOSIS FUNGOIDES

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

Abstract

The review highlights the current understanding of the epidemiology, etiology, pathogenesis, existing classifications of mycosis fungoides. Methods for diagnosis and treatment of the pathology are described, among which photodynamic therapy (PDT) plays an important role. The main advantages of PDT for mycosis fungoides include the absence of systemic toxicity, non-invasiveness, selectivity, absence of carcinogenic potential, the possibility of repeated courses of treatment, and good cosmetic results. This review collects and analyzes the results of clinical trials of PDT in patients with mycosis fungoides. The analysis showed high efficiency of PDT in patients with mycosis fungoides with isolated or limited spots and plaques. PDT can be considered as the therapy of choice in patients with facial lesions when a good cosmetic result is one of the main requirements, and radiation therapy, nitrogen mustard or carmustine can leave permanent and visible scars. Plaques located in the axillary or inguinal skin folds that are inaccessible to phototherapy can also be treated with PDT.

Key words: photodynamic therapy, mycosis fungoides, 5-aminolevulinic acid.

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

ФОТОДИНАМИЧЕСКАЯ ТЕРАПИЯ В ЛЕЧЕНИИ БОЛЬНЫХ ГРИБОВИДНЫМ МИКОЗОМ

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

Резюме

В обзоре освещены современные представления об эпидемиологии, этиологии, патогенезе, существующих классификациях грибовидного микоза. Описаны методы диагностики и лечения патологии, важную роль среди которых играет фотодинамическая терапия (ФДТ). К основным преимуществам ФДТ грибовидного микоза относятся отсутствие системной токсичности, неинвазивность, избирательность действия, отсутствие канцерогенного потенциала, возможность проведения повторных курсов лечения и хорошие косметические результаты. В настоящем обзоре собраны и проанализированы результаты клинических исследований ФДТ пациентов с грибовидным микозом. Анализ показал высокую эффективность ФДТ у пациентов с грибовидным микозом с изолированными или ограниченными пятнами и бляшками. ФДТ можно рассматривать как терапию выбора у пациентов с очагами на лице, когда одним из основных требований является хороший косметический результат, а лучевая терапия, азотный иприт или кармустин могут оставить постоянные и видимые шрамы. Бляшки, расположенные в подмышечных или паховых складках кожи, недоступные для фототерапии, также можно лечить с помощью ФДТ.

Ключевые слова: фотодинамическая терапия, грибовидный микоз, 5-аминолевулиновая кислота.

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

Mycosis fungoides (MF) is a primary epidermotropic T-cell lymphoma of the skin, characterized by the proliferation of small and medium T-lymphocytes with cerebriform nuclei. MF is one of the most common forms of cutaneous T-cell lymphomas. In the earliest stages of the disease, patients usually present with isolated skin lesions resembling eczema or extensive erythema [1]. The term "mycosis fungoides" was introduced by J.L.M. Alibert in 1832 in relation to the unusual skin rash he described in 1806, which developed into tumors in the form of mushrooms [2].

Epidemiology

After the gastrointestinal tract, the skin is the second most common site of extranodal non-Hodgkin's lymphoma, with an estimated annual incidence of 1 case per 100,000 population. According to the fourth edition of the WHO Blue Book, MF is the most common form of primary cutaneous lymphomas (skin lymphomas without evidence of extracutaneous disease at the time of diagnosis) according to the classification of skin tumors. The proportion of MF in the overall incidence of primary cutaneous lymphomas is 53% of primary cutaneous T-cell lymphomas and 45% of all primary cutaneous lymphomas (of which 39% is the classic form of MF, 6% are its variants according to the WHO-EORTC classification, including folliculotropic MF – 5%, pagetoid reticulosis – less than 1%, granulomatous loose skin syndrome – less than 1%) (Table 1). Another 20% of primary cutaneous cutaneous lymphomas are due to primary cutaneous $CD30^+$-lymphoproliferative diseases, including 8% to primary cutaneous anaplastic large cell lymphoma° 12% to lymphomatoid papulosis; 6% for primary cutaneous $CD4^+$ T-cell lymphoproliferative disease of small and medium cells; 2% for Cesari's syndrome; 9% – for primary B-cell lymphoma of the marginal zone of the skin; 12% – for primary cutaneous lymphoma from the cells of the follicular center; 4% – for primary cutaneous diffuse large B-cell lymphoma, lower extremity type. The incidence of other types of primary cellular lymphomas is less than 1% for each type [3, 4].

Classification

In 1876, Bazin described three classical stages in the development of MF: macules, infiltrated plaques, and tumors. Each of them differs from the previous one by increasing thickness. Stages can overlap or register at the same time. If only tumors are present, with no preceding or coexisting macules or plaques, the diagnosis of MF should be questioned. WHO and the European Organization for Research and Treatment of Cancer, in addition to the classical form of Albert-Bazen MF, characterized by the development of spots, plaques and tumors, distinguish three variants of the disease: folliculotropic MF, pagetoid reticulosis, and granulomatous flaccid skin syndrome [1, 2, 5].

Clinical picture

MF usually presents clinically as persistent patches or plaques that are sometimes itchy and usually affect areas of the skin that are not exposed to sunlight. These lesions can often remain unchanged for many years or even throughout the life of the patient, but progression to disseminated lesions, tumor development, or lesions of internal organs can occur, which worsens the prognosis and requires systemic treatment [6]

Diagnosis

Diagnosis of MF is usually based on characteristic clinical manifestations and confirmed by routine histology [2]. The gold standard is light microscopy of affected skin sections stained with hematoxylin-eosin, but early diagnosis can be difficult because the initial histological picture may resemble other chronic inflammatory dermatoses [1, 2]. To date, there are no diagnostically significant molecular markers that can reliably distinguish between malignant and benign T cells [1]. The histological picture is more often diagnostically significant at the plaque stage. In this case, histology reveals a streak-like or lichenoid infiltrate of mononuclear cells in the papillary dermis with overlying epidermotropism (intraepidermal lymphocytes with slight spongiosis). These lymphocytes are found individually or in clusters within the epidermis, often surrounded by a clear halo (Pauttier microabscesses). When examining mononuclear cells at high magnification, hyperchromatic and irregular contours of the nuclei are revealed. The picture of psoriasiform hyperplasia of the epidermis with hyperkeratosis and focal parakeratosis is determined [2].

Traditional treatment

Most cases of MF are characterized by a sluggish course with a low risk of disease progression, but complete regression is rarely achieved. Therefore, the main goal of treatment is to minimize symptomatic morbidity and limit disease progression [1].

The variability of the clinical picture and the clinical course of MF has led to the emergence of many schemes and complex treatment algorithms. It is important to understand that very little clinical research has been done on MF to date, as it is a fairly rare condition and the evidence on which current treatment recommendations are based is limited [6].

Treatment methods used in MF can be divided into topical, skin-directed, and systemic therapies.

For the treatment of stage IA, IIA, IIB MF, 2 types of skin-directed therapy are traditionally used: for a localized form (IA) and for a widespread (IIA, IIB) lesion (Table 1).

The most common treatment for the early stages of MF (stages IA-IIA) are topical corticosteroids [6, 14]. Most often they are used as an adjunct to other

Таблица 1
Рекомендации по лечению грибовидного микоза IA, IIA, IIB стадии [6,10-13]
Table 1
Treatments for early mycosis fungoides (stages IA, IIA, IIB) [6,10-13]

Локализованная форма Localized disease	Распространенная форма Widespread disease
Местное применение кортикостероидов Topical corticosteroids	Местное применение кортикостероидов Topical corticosteroids
Местная химиотерапия (азотный иприт, кармустин) Topical chemotherapy (nitrogen mustard, carmustine)	Местная химиотерапия (азотный иприт, кармустин) Topical chemotherapy (nitrogen mustard, carmustine)
Локальная лучевая терапия Local radiotherapy	Фототерапия: UVB, псорален–UVA Phototherapy: UVB, psoralen–UVA
Местное применение ретиноидов (бексаротен, тазаротен) Topical retinoids (bexarotene, tazarotene)	Тотальная кожная электронно-лучевая терапия Total electron-beam skin therapy
Фототерапия: UVB, псорален–UVA Phototherapy: UVB, psoralen–UVA	
Местное применение имиквимода Topical imiquimod	

local and systemic treatments at all stages. They induce apoptosis, affect the adhesion of lymphocytes to the endothelium and suppress transcription factors (nuclear factor-kB and activator protein-1), reduce the production of cytokines, adhesion molecules and growth factor. Nitrogen mustard therapy and phototherapy are also often used, which, according to some authors, have the same efficacy in early MF and are used in combination with additional maintenance therapy necessary to achieve long-term complete remissions. Phototherapy (narrow-band UVB or psoralen-UVA) alone or in combination with systemic therapy (bexarotene, interferon, or methotrexate) is effective for widespread plaques [6]. PUVA therapy demonstrates low efficiency when used at the stage of the tumor or erythrodermic and folliculotropic MF; however, studies show its effectiveness in combination with low doses of systemic agents, such as interferon-alpha [14, 15]. Total skin electron beam therapy at a standard dose of 30 Gy is an effective treatment for obstinate/recurrent extensive plaques and fungal tumor mycoses, but is associated with significant dermal toxicity. This type of therapy reduces the number of circulating abnormal T cells that pass through the vasculature of the dermis and are radiosensitive. However, there are conflicting reports on its efficacy in erythrodermic MF. For isolated lesions, low-dose local radiation therapy may also be useful [14].

Systemic therapy of MF is carried out both separately and in the form of complex treatment. If skin-targeted therapy does not provide an adequate response or in cases of advanced disease, single-component systemic therapy (eg, bexarotene) is prescribed. Immunomodulators such as interferons and retinoids are also commonly used as first-line monotherapy for advanced forms of MF, as well as in low dose combinations with topical agents. Histone deacetylase inhibitors (vorinostat or romidepsin) are also effective agents for monotherapy in cutaneous and nodular forms of MF and forms of the disease with blood involvement. Alemtuzumab is an effective drug for erythrodermic mycosis fungoides with a deficit of central memory T cell population. Chemotherapy is usually used to treat obstinate or rapidly progressive MF. In advanced forms of MF, allogeneic stem cell transplantation can be effective [14].

Many of the traditional MF therapies described above show insufficient efficacy and are accompanied by long-term side effects. This is especially true of lesions located in places such as the palms and soles of the feet. This necessitates the search for alternative treatment options for mycosis fungoides.

Photodynamic therapy

PDT is an effective and non-invasive method of exposure with good clinical results [16].

The mechanism of photodynamic effects in MF is not fully understood. It is possible that, in addition to the direct destruction of pathological lymphocytes due to the generation of reactive oxygen species, the inflammatory response induced by PDT contributes to the therapeutic effect [17-20].

In 1994, Boenhcke et al. demonstrated how PDT inhibits lymphocyte proliferation in MF plaques both *in vivo* and *in vitro* [21].

M. Lam et al. studied the mechanisms of photocytotoxicity of the silicon-phthalocyanine photosensitizer Pc 4 in relation to pathological T-lymphocytes in the skin cell culture of patients with MF. Studies have shown that PDT with PC 4 predominantly induces apoptosis of $CD4^+$ $CD7^-$ transformed T-lymphocytes in the blood compared to $CD11b^+$ monocytes and normal T-cells, that is, selectively affects pathological T-lymphocytes. It was also shown that PDT with Pc4 reduced the level of the antiapoptotic protein Bcl-2 [22].

The high efficiency and absence of systemic toxicity of PDT are due to the selective accumulation of photosensitizers in the lesions and local exposure to light [17].

For PDT of MF, preparations based on 5-aminolevulinic acid (5-ALA) and 5-ALA methyl ester are usually used. 5-ALA methyl ester has more pronounced lipophilic properties, shorter incubation time and higher selectivity for pathological lymphocytes compared to 5-ALA. Both of these drugs are metabolized in the human body with the formation of photoactive protoporphyrin IX [17].

The selectivity of the accumulation of protoporphyrin IX in pathological lymphocytes in the foci of MF is due to a number of factors. Protoporphyrin IX is more actively synthesized in transformed lymphocytes. This is due to excessive iron intake due to increased expression of the CD71 transferrin receptor [23], increased activity of porphobilinogen deaminase, an enzyme that limits the rate of heme synthesis [24], and acceleration of the cell cycle in cells, which increases their ability to absorb 5-ALA [6, 25].

In addition, changes in the stratum corneum of the epidermis in plaques contribute to the penetration of the photosensitizer into the skin [6, 26].

Evaluation of the effectiveness of PDT is carried out on the basis of a clinical picture demonstrating the disappearance of MF foci, and a histological examination. A number of authors believe that the assessment of the clinical response is sufficient and reflects the real effectiveness of the treatment. According to other researchers, in evaluating the effectiveness of MF treatment, the histological therapeutic response is more important than the clinical response.

Histological response to treatment has been evaluated in a number of studies, but the small number of observations and conflicting data obtained by different investigators do not allow an unambiguous conclusion to be made about the need to confirm clinical response by histological examination.

R. Ammann et al. and E. Diez-Recio et al. reported a complete histological treatment and the absence of atypical lymphocyte infiltrates in all MF plaques after PDT with 5-ALA in 3 patients whose clinical outcome was assessed as complete regression. Both groups of researchers described pigmentary changes with the presence of melanophages, skin fibrosis, epidermal atrophy, and residual lymphocytes in the infiltrate [27, 28].

Concurrently S.T. Kim et al. believe that the final decision to assess the effect of treatment as a complete or partial regression should be determined by the results of histological analysis [17]. According to the results obtained, 7 out of 10 patients with unilateral MF showed an obvious improvement in their condition. Most previous studies did not follow up histologically. In the described study, 8 out of 10 patients underwent histological observation, and only 5 of them had a clinical and histological response that coincided and could be assessed as a complete regression. In 2 patients with full therapeutic clinical effect, the histological report showed partial regression of the lesions. D.W. Edstrom et al. also found a residual infiltrate of atypical lymphocytes in 2 patients after PDT with 5-ALA in lesions that showed a complete clinical response [29]. Possibly, these results are related to the insufficient depth of light penetration for selective action on lymphocytes in MF plaques. D. Eich et al. treated 8 patients with tumor form of MF. A subsequent biopsy showed the absence of histological signs of GM in the infiltrate up to a depth of 1.5 mm, while atypical lymphocytes continued to be detected in deeper tissues [30]. Published data do not clearly prove that with complete clinical regression of MF foci after PDT, atypical lymphocytes in the foci were completely eliminated, and therefore patients with a complete clinical response after PDT should be under dynamic monitoring for timely detection of a possible relapse [6].

Table 2 summarizes the results of the main clinical studies presented in the Pubmed database evaluating the effectiveness of PDT for MF. These studies include a total of data from 71 patients with 120 MF lesions treated with PDT from 1994 to 2018. Most of the authors used red light sources (coherent and incoherent). In earlier works, preparations based on 5-ALA were used. Since 2006, most studies have used 5-ALA methyl ester. The number of PDT courses varied from 1 to 9. A light dose of 37 J/cm^2 was predominantly used; in some cases, the light dose during irradiation was higher and reached 200 J/cm^2.

The conducted studies show the promise of using PDT as one of the options for localized skin-targeted therapy. In the late stages of advanced MF (stage IIB and above), PDT is of less interest due to the complexity of influencing the generalized process; however, it can be used as a treatment for individual plaques or tumors [6].

Due to the small number of observations and the

lack of large-scale clinical studies of the effectiveness of PDT in patients with MF, the optimal modes of PDT have not yet been determined [17]. It can be noted that the high efficiency of treatment was achieved using drugs based on both 5-ALA and 5-ALA methyl ester. The light dose and the frequency of courses varied significantly in different studies, and therefore it is difficult to determine whether there is a correlation between these indicators and the effectiveness of treatment, although most authors indicated the need for several courses of PDT.

Patients generally tolerate PDT well, although most studies report a slight burning sensation during the procedure. After PDT, some patients developed erythema and edema at the treatment site, much less often – scabs and erosion. Studies have shown that lesions can develop mild pigmentary changes, most often hyperpigmentation or hair loss [16, 17, 27-29, 31-43].

There have been several studies evaluating the effectiveness of fluorescence diagnosis of MF.

The fluorescence pattern of MF foci was described by A. Orestein et al. as weak and diffuse in spots, intense with well-defined boundaries in plaques and tumors [34]. The authors reported that when irradiated during a PDT session, the fluorescence intensity decreased faster in spots and plaques than in tumors.

In the studies of Svanberg K. et al. the fluorescent contrast of MF foci after exposure to 20% exposure with 5-ALA was 5.0 [31].

Таблица 2
Сводные данные результативности применения фотодинамической терапии у пациентов с грибовидным микозом
Table 2
Summary of the effectiveness of photodynamic therapy in patients with mycosis fungoides

Авторы Authors	Количество очагов/ пациентов No. of lesions/ No. of patients	Фотосенсибилизатор Photosensitizer	Режим облучения Irradiation mode	Световая доза, Дж/см² Light dose, J/cm²	Количество курсов ФДТ Number of PDT courses	Тип очага Type of lesion	Результат терапии, % пациентов Results of therapy, % of patients	Срок наблюдения, мес Follow-up period, month
Svanberg и соавт., 1994 [31]	4/2	5-АЛК 5-ALA	Лазер, 630 нм Laser, 630 nm	60	1-2	Не указан Not specified	ПР 50% (1/2) CR 50% (1/2)	Не указан Not specified
Wolf и соавт., 1994 [32]	2/2	5-АЛК 5-ALA	Видимый свет Visible light	40	4-5	Бляшка Plaque	ПР 100% CR 100%	3–6
Amman и соавт., 1995 [27]	1/1	5-АЛК 5-ALA	Видимый свет Visible light	Не указана Not specified	1	Бляшка Plaque	CR 100%	Не указан Not specified
Wang и соавт., 1999 [33]	3/1	5-АЛК 5-ALA	Лазер, 635 нм Laser, 635 nm	60	3-4	Бляшки Plaques	CR 100%	17-33
Markham и соавт., 2001 [35]	1/1	5-АЛК 5-ALA	Некогерентный источник света, 580-740 нм Noncoherent light, 580–740 nm	20	5	Опухоль Tumor	ПР 100% CR 100%	12 В течение 12 мес наблюдения возник рецидив (пятно и бляшка). During the 12 months of follow-up, a relapse occurred (patch and plaque).
Edstrom и соавт., 2001 [29]	12/10	5-АЛК 5-ALA	Некогерентный источник света, 600-730 нм Noncoherent light, 600–730 nm	33-180	2-11	10 бляшек 2 опухоли 10 plaques 2 tumors	**Бляшки:** ПР 70% (7/10) ЧР 20% (2/10) не оценен 10% (1/10) **Опухоли:** отсутствие эффекта 100% (2/2) **Plaques:** CR 70% (7/10) PR 20% (2/10) not rated 10% (1/10) **Tumors:** no effect 100% (2/2)	4–19

Авторы Authors	Количество очагов/ пациентов No. of lesions/ No. of patients	Фотосенсибилизатор Photosensitizer	Режим облучения Irradiation mode	Световая доза, Дж/см² Light dose, J/cm²	Количество курсов ФДТ Number of PDT courses	Тип очага Type of lesion	Результат терапии, % пациентов Results of therapy, % of patients	Срок наблюдения, мес Follow-up period, month
Leman и соавт., 2002 [36]	2/1	5-АЛК 5-ALA	Лазер, 630 нм Laser, 630 nm	100	4	Пятна Patches	ПР 100% CR 100%	12
Coors и соавт., 2004 [37]	7/4	5-АЛК 5-ALA	Некогерентный источник света, 60-160 нм Noncoherent light, 60–160 nm	72-144	1-7	Бляшки Опухоли Plaques Tumors	ПР 100% CR 100%	14–18
Zane и соавт., 2006 [38]	5/5	Метиловый эфир 5-АЛК 5-ALA methyl ester	Лазер, 635 нм Laser, 635 nm	37,5	1-9	Пятна Patches	ПР 80% (4/5) ЧР 20% (1/5) CR 80% (4/5) PR 20% (1/5)	12–34
Díez-Recio и соавт., 2007 [28]	2/2	5-АЛК 5-ALA	Импульсный лазер на красителе, 585 нм Pulsed dye laser, 585 nm	8 Дж/см², длительность импульса 0,45 мс, степень перекрытия импульсов 1 Гц, количество импульсов 8-22 8 J/cm², pulse duration of 0.45 ms, degree of pulse overlap of 1 Hz 8-22 pulses per treatment	3	Бляшки Plaques	ПР 100% CR 100%	34
Hegyi J. и соавт., 2008 [42]	1/1	Метиловый эфир 5-АЛК 5-ALA methyl ester	Светодиод, 630 нм LED, 630 nm	100-200	3	Пятно Patche	ПР 100% CR 100%	16
Orenstein и соавт., 2009 [34]	6/2	5-АЛК 5-ALA	Некогерентный источник света, 580-720 нм Noncoherent light, 580–720 nm	170 (пятно) 380 (опухоли) 170 (patch) 380 (tumors)	1 (пятно) Фракционное облучение (опухоли) 1 (patch) Fractional irradiation (tumors)	1 пятно 5 опухолей 1 patch 5 tumors	ПР 100% CR 100%	24-27
Fernández-Guarino и соавт., 2010 [39]	24/12	Метиловый эфир 5-АЛК 5-ALA methyl ester	Лазер, 630 нм Laser, 630 nm	37	В среднем 5,7 Mean 5,7	Бляшки Plaques	ПР 50% (6/12) ЧР 42% (5/12) CR 50% (6/12) PR 42% (5/12)	6–36
Kim и соавт., 2012 [17]	16/10	Метиловый эфир 5-АЛК 5-ALA methyl ester	Светодиод, 630 нм LED, 630 nm	37,5	2-6	6 пятен 10 бляшек 6 patches 10 plaques	**По очагам:** ПР 13% (2/16) ЧР 31% (5/16) отсутствие эффекта 56% (9/16) CR 13% (2/16) PR 31% (5/16) no effect 56% (9/16) **Или по пациентам:** ПР 20% (2/10) ЧЭ 50% (5/10) отсутствие эффекта 30% (3/10) CR 20% (2/10) PR 50% (5/10) no effect 30% (3/10)	8-31

Авторы Authors	Количество очагов/ пациентов No. of lesions/ No. of patients	Фотосенсибилизатор Photosensitizer	Режим облучения Irradiation mode	Световая доза, Дж/см² Light dose, J/cm²	Количество курсов ФДТ Number of PDT courses	Тип очага Type of lesion	Результат терапии, % пациентов Results of therapy, % of patients	Срок наблюдения, мес Follow-up period, month
Quéreux и соавт., 2013 [40]	29/12	Метиловый эфир 5-АЛК 5-ALA methyl ester	Лазер, 630 нм Laser, 630 nm	37	2-6	20 пятен 9 бляшек 20 patches 9 plaques	ПР 50% (6/12) ЧР 25% (3/12) отсутствие эффекта 25% (3/12) CR 50% (6/12) PR 25% (3/12) no effect 25% (3/12)	6-35
Kaufmann и соавт., 2017 [16]	1/1	Метиловый эфир 5-АЛК 5-ALA methyl ester	Светодиод, 630 нм LED, 630 nm	Не указана Not specified	8	Пятно Patche	ПР 100% CR 100%	48
Pileri и соавт., 2017 [41]	4/4	Метиловый эфир 5-АЛК 5-ALA methyl ester	Диодная лампа, 630 нм LED lamp, 630 nm	37	4-9	Пятна Patches	ПР 50% (2/4) ЧР 50% (2/4) CR 50% (2/4) PR 50% (2/4)	6-120
Jang и соавт., 2018 [43]	2/2	Метиловый эфир 5-АЛК 5-ALA methyl ester	Светодиод, 630 нм LED, 630 nm	37,5	2	Пятна Patches	ПР 100%	47-87

Примечания: ПР – полная регрессия, ЧР – частичная регрессия.
Note: CR – complete regression, PR – partial regression.

Conclusion

The main advantages of PDT in patients with MF include the absence of systemic toxicity, non-invasiveness, selectivity of action, the absence of carcinogenic potential, the possibility of conducting several courses of treatment, and good cosmetic results. PDT shows high efficiency in patients with isolated or limited spots and plaques. PDT can be considered as the therapy of choice in patients with facial lesions where a good cosmetic result is one of the main requirements, while radiation therapy, nitrogen mustard or carmustine can leave permanent and visible scars. Plaques located in the axillary or inguinal folds of the skin, which are inaccessible to phototherapy, can also be treated with PDT [6].

Studies show that several courses of PDT are usually required to treat patients with MF, although the exact frequency of courses remains uncertain. Most of the authors conducted courses every 2-4 weeks with a frequency that allows removing crusts before the next course. The total number of courses depends on the clinical response and varies significantly in the studies of different authors (range from 2 to 11 courses) [16, 17, 27-29, 31-43]. According to M. Fernández-Guarino et al. in the absence of a response to PDT, 6 courses may be considered sufficient before interrupting treatment and considering other therapy options [6].

Treatment failure in patients with large plaques has been reported in some cases, and therefore the authors do not recommend PDT for lesions greater than 7.5 cm in diameter [6]. Other clinical studies show that the effectiveness of PDT does not depend on the size of the lesion, but may be related to the number of lesions, the thickness of the stratum corneum, the tumor cells infiltration degree, and the depth of invasion [17].

Despite a small number of studies and the absence of clear recommendations on PDT regimens in patients with MF, PDT is an effective and promising treatment for these patients.

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ACTINIC KERATOSIS (review of literature)

Filonenko E.V.[1], **Okushko S.S.**[2]
[1]P.A. Herzen Moscow Oncology Research Center – branch of FSBI NMRRC of the Ministry of Health of the Russian Federation, Moscow, Russia
[2]The Federal State-Financed Institution Skobelkin State Scientific Center of Laser Medicine under the Federal Medical Biological Agency, Moscow, Russia

Abstract

Actinic keratosis is an important medical and social problem, the correct diagnosis and treatment of which will help to avoid the development of invasive forms of cutaneous squamous cell carcinoma. With the further development of the early diagnosis of cancer, including skin cancer, the increase in human life expectancy, and the popularization of travel to exotic countries, the number of cases of actinic keratosis among the population will continue to grow. In this regard, it is important to discuss the causes and pathogenesis of the disease, the varied clinical picture of the disease, methods of non-invasive diagnostics, as well as methods of treatment, of which there are a great many in the treatment of actinic keratosis today. However, each of the methods has both advantages and disadvantages, and in the global trend towards a personalized approach to treatment, it is important to choose from the standpoint of evidence-based medicine the most suitable for each individual patient. Moreover, after treatment of actinic keratosis, relapses often occur, which are the result of insufficient diagnosis and the development of incorrect treatment tactics. The review article provides the clinical picture of actinic keratosis, diagnostic and therapeutic methods, and their comparison with each other in terms of efficacy and safety.

Key words: actinic keratosis, cutaneous squamous cell carcinoma in situ, photodynamic therapy.

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

АКТИНИЧЕСКИЙ КЕРАТОЗ (обзор литературы)

Е.В. Филоненко[1], **С.С. Окушко**[2]
[1]«Московский научно-исследовательский онкологический институт им. П.А. Герцена – филиал ФГБУ «Национальный медицинский исследовательский центр радиологии» Министерства здравоохранения Российской Федерации, Москва, Россия
[2]«Государственный научный центр лазерной медицины им. О.К. Скобелкина ФМБА», Москва, Россия

Резюме

Актинический кератоз (АК) рассматривается как предраковое состояние, ассоциированное с повышенным, в случаях отсутствия лечения до 20%, риском развития плоскоклеточного рака кожи. По мере дальнейшего развития технологий ранней диагностики онкологических заболеваний, в том числе и рака кожи, увеличения продолжительности жизни людей и популяризации путешествий в экзотические страны, число случаев АК среди населения будет возрастать. В этой связи информирование медицинского сообщества о причинах и патогенезе заболевания, разнообразии клинической картины АК, методах неинвазивной диагностики и лечения представляется важной медико-социальной задачей. Каждый из диагностических и терапевтических методов имеет свои преимущества и недостатки, поэтому, руководствуясь данными доказательной медицины, важно персонифицировать подходы к диагностике и лечению для каждого конкретного пациента. Более того, после лечения АК часто возникают рецидивы, которые являются следствием недостаточной диагностики и выработки неправильной тактики лечения. В обзорной статье приводятся клиническая картина АК, сравнительная характеристика диагностических и лечебных методов с позиций эффективности и безопасности применения у пациентов с АК.

Ключевые слова: актинический кератоз, плоскоклеточный рак кожи in situ, фотодинамическая терапия.

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

Introduction

The global trend of increasing life expectancy and aging of the population, along with the improvement of early diagnosis of malignant neoplasms, contribute to the growth of cancer incidence. Technological progress, the development of air transport and the popularization of travel to exotic countries lead to the fact that more and more people with fair skin find themselves in geographic areas with excessive insolation. Long-term exposure to ultraviolet radiation is a major cause of melanoma and non-melanocytic skin cancers (NMSCs), such as squamous cell skin cancer (SCSC), basal cell skin cancer (BCSC), Merkel cell carcinoma, and other rarer skin cancers. Most malignant skin neoplasms develop against the background of diseases classified as "precancerous skin lesions". Actinic keratosis (AK), also known as solar keratosis or senile keratosis, is a premalignant skin lesion composed of proliferating atypical epidermal keratinocytes that can progress to invasive squamous cell carcinoma [1].

Etiology and pathogenesis

AK was described by Dubreuil in 1826 [2]. Freudenthal later proposed the term "senile keratoma", and in 1958 Pinkus renamed these lesions AK [3]. Some authors suggest considering them as in situ neoplasms, since they originate from clonal DNA modifications in keratinocytes [3]. A number of authors consider AK as a dermatosis with signs of malignancy from the moment of their occurrence. In terms of cytological changes, they are epidermal keratinocytes similar to those seen in SCSC, including loss of polarity, nuclear pleomorphism, maturation dysregulation, and increased mitosis; from the molecular point of view, they are identical mutations in the p53 protein [4]. According to the classification of skin neoplasms of the World Health Organization (WHO), AK is classified as skin precancer [5].

Excessive exposure to UV radiation is the main factor acting as a complete carcinogen, causing and promoting tumor growth [6]. UV radiation activates molecular signaling cascades that lead to changes in regulatory cytokine levels, immunosuppressive effects, abnormal cell differentiation, and apoptosis. UV radiation is divided into UV-A, UV-B and UV-C radiation. About 94-97% of the total UV radiation that reaches the Earth's surface consists of UV-A rays. UV-B rays are partially filtered by the ozone layer of the atmosphere and make up 3-6%, UV-C rays are almost completely absorbed by the atmosphere and only their minimum levels reach the Earth's surface.

UV-A radiation (wavelength 320-400 nm) penetrates deep into the skin and stimulates the production of reactive oxygen species that damage cell membranes, nuclei and protein molecules [6], promotes mutational substitutions of guanine (G) for thymine (T) in DNA [7]. As a result, signal transduction and cellular interaction pathways are affected, which contributes to abnormal cell proliferation [6].

UV-B radiation (290-320 nm) is absorbed by cellular DNA, causing errors in the repair of cyclobutane-pyrimidine dimers and the formation of pyrimidine photoproducts, as well as characteristic cytosine-thymine (C-T) DNA substitutions [6]. These effects lead to mutations in the p53 protein, which regulates the cell cycle and repairs damaged DNA, mutations in the telomerase gene, and increased production of pro-inflammatory cytokines [4, 6].

The mechanisms involved in the occurrence of AK include inflammation, oxidative stress, immunosuppression, impaired apoptosis, cell cycle deregulation and cell proliferation, and tissue remodeling [6]. The inflammatory process is mediated by the metabolic breakdown of arachidonic acid through the production of pro-inflammatory cytokines, mast cell activation, and macrophage migration inhibition factor. As a result of the activation of these mediators, lipid peroxidation, an increase in intralesional levels of T-lymphocytes and Langerhans cells, an increase in p53 and Bcl-2, and a decrease in Fas (cd95) and Fas-ligand, which are important initial factors in the process of apoptosis of UV-mutated cells, are turned on. An association between inflammation and the development of AK is seen in lesions progressing to SCSC. This is supported by the fact that anti-inflammatory therapies are effective in the treatment of AK [6]. Oxidative stress is also involved in the process of carcinogenesis as a result of excessive exposure to UV radiation, which leads to the production of reactive oxygen species and ends with lipid peroxidation and cell destruction with damage to genomic and mitochondrial DNA [6]. Altered cell signal transduction pathways result from membrane tyrosine kinase phosphorylation, changes in epidermal growth factor in Ras and RAF, and in the dissociation of nuclear factor B from inhibitory complex B [6]. These events lead to the production of cytokines, including interleukin-1 (IL-1), tumor necrosis factor and IL-6, as well as activation of the metabolic breakdown of arachidonic acid. The end result is signal transduction of transcription factors into cell nuclei with modifications in gene expression [4].

The difficulty in establishing unambiguous criteria for determining when an AK undergoes transformation into SCSC supports this hypothesis. According to Ackerman, there is no clear threshold between AK and initial SCSC, and AK is considered part of the evolutionary spectrum of SCSC, described as "embryonic" SCSC. Therefore, the proposed nomenclature to replace the term "actinic keratosis" would include keratinocytic intraepidermal neoplasia and intraepidermal solar keratotic SCSC [3]. AK is formed as a result of the proliferation of keratinocytes with varying degrees of dysplasia in the epidermis, moreover, they have the potential

for malignant transformation, especially in the case of SCSC, lesions occur predominantly in areas exposed to the sun [2].

The likelihood and rate of transition from AK to SCSC is individual, highly variable and unpredictable. A systematic review noted a high rate of progression of 0.53% per lesion per year in patients with a history of SCSC or other SCC. Solitary AK has been found to have a high regression rate of 15 to 53% per year, as well as a long-term recurrence rate of over 50%. In the review article, this trend was partly explained by the methodological shortcomings of published studies: the lack of data on the treatment and prevention of AK using sunscreens, and a high percentage of patients dropping out of the study. However, literature data and clinical experience do show that AK and light-induced skin damage are a dynamic rather than a static pathological process [8].

The cumulative dose of UV exposure is a major risk factor for the development of AK and SCSC. If a patient has six or more lesions of AK, SCSC, or an area of skin with "field cancerization" (the so-called field of cancerization or tumor field) with an area of \u200b\u200bat least 4 cm^2 that is exposed to UV radiation due to the patient's work activity, the presence of possible occupational disease.

Epidemiology

AK ranks third in frequency of dermatological consultations, second only to acne and dermatitis [9]. With the overall population aging, a gradual increase in the incidence of AK is expected. With regard to the prevalence of AK, according to WHO estimates, the highest levels are observed in representatives of the Caucasian race living near the equator [10]. The prevalence of AK increases with increasing age of patients: in Caucasians, from less than 10% at the age of 20-29 years to 80% in the group of people 60-69 years old [4].

In the Russian Federation, skin cancer ranks first or second in the overall (both sexes) structure of the incidence of malignant neoplasms after breast cancer. According to statistics, the incidence of non-melanoma skin cancer (NMSC) is steadily increasing: from 236.5 cases per 100,000 population in 2009 to 310.4 cases in 2019. In 2019, 73,150 patients were registered with a diagnosis of skin cancer (except melanoma) for the first time in their lives [11]. Given the increase in the incidence of skin cancer in the population, timely diagnosis, treatment and prevention of AK is an urgent problem.

Clinical and histological picture

AK appears as erythematous macules, papules, or plaques, usually with poorly defined borders, which may be covered with dry pells. Sometimes they are better identified by palpation than by visual inspection, they can represent hyperkeratosis of varying degrees [2, 12]. Lesions may be solitary or multiple, and the color may vary from pink to erythematous or brownish in the case of pigmented AK [13, 14]. The degree of infiltration also varies depending on the intensity and degree of dysplasia or associated inflammation. In most cases, AK occurs without additional symptoms, although some patients experience discomfort, burning, pain, bleeding, and itching [2, 12, 19]. AK predominantly occurs on areas of the skin that are chronically exposed to light, such as the face, bald scalp, neck, shoulders, forearms, and back of the hands [4]. In both sexes, lesions most often occur on the upper extremities, as well as on the face and scalp. These areas, especially the head, neck, and forearms, are responsible for 75% of reported lesions. AK can manifest itself in various forms and existing clinical variants, such as hyperkeratotic AK, atrophic, pigmental lichenoid actinic keratosis, cutaneous horn and actinic cheilitis [14].

The gold standard of the AK assessment system in clinical practice has not yet been defined. It is important to emphasize that the degree of agreement between clinical and histological gradation is low, which confirms the need to treat all AK foci, regardless of their severity [15]. In a number of literary sources, based on histological examination, AK is divided into seven subtypes: hypertrophic, atrophic, bowenoid, acantholytic, epidermolytic, lichenoid, and pigmental [2, 4, 16]. All histological subtypes can be seen in a single lesion [4].

Diagnosis

AK in most cases is diagnosed clinically. Lesions that are identified during the physical examination and confirmed by the history can be recognized and do not require additional examination. Dermoscopy has been shown to be extremely important in increasing the level of certainty and accuracy in questionable lesions. Other non-invasive imaging modalities such as confocal microscopy (CM) may also be useful in certain situations where available. In questionable cases, histological examination is required to confirm the diagnosis.

Treatment

Given that AK is potentially associated with malignancy and it is impossible to predict which lesions will transform and which will regress, all lesions should be treated [2, 17]. When managing patients with AK, it is necessary to regularly examine the skin of the whole body with an assessment of the presence of tumor fields and the therapeutic effect on them, the use of ablative treatment methods for hyperkeratotic lesions, informing patients about the chronic course of the disease, the need for photoprotection and periodic repetition of treatment procedures, and regular self-examination of the skin by the patient [18]. The

impact on AK foci includes several methods. Basically, they can be divided into ablative or surgical methods and topical therapy by non-surgical methods (see Table) [19].

The use of these methods in combination or sequentially in the management of such patients is a common practice [20]. It is noteworthy that from 25 to 75% of treated patients need re-treatment within 12 months due to the appearance of new lesions, which indicates a chronic course of AK, even if the tumor fields were treated [21]. Most often, relapses are observed in patients who have undergone only cryotherapy, less often in those who have received treatment with an effect on tumor fields [22]. A large systematic review analyzing various treatments for AK concluded that 5-fluorouracil (5-FU), diclofenac, imiquimod, and ingenol mebutate (IM) may have similar efficacy [23].

The choice of treatment varies depending on the clinical picture, the location of AK foci, their number and severity of the lesion; therefore, treatment should be selected individually in accordance with the individualities of each patient. Techniques aimed at the lesion focus, as a rule, can be applied quickly, do not require long-term rehabilitation, but they are effective only with lesions limited in area. The application of methods aimed at the tumor field requires long-term treatment from several days to months and, therefore, requires high discipline and commitment from the patient.

Таблица
Абляционно-хирургические и нехирургические методы лечения актинического кератоза
Table
Ablative-surgical and non-surgical treatments for AK

Варианты хирургического лечения Surgical treatment options	Местные и пероральные методы лечения Topical and oral treatments
Криохирургия (уровень убедительности рекомендаций А, уровень достоверности доказательств 1 ++) [20] Cryosurgery (recommendation strength level A, level of evidence 1++) [20]	5-Фторурацил (уровень убедительности рекомендаций А, уровень достоверности доказательств 1 ++) [20] 5-Fluorouracil (recommendation grade A, evidence level 1++) [20]
CO_2-лазер (уровень убедительности рекомендаций В, уровень достоверности доказательств 1+) [20] CO_2 laser (recommendation grade B, evidence level 1+) [20]	Имиквимод (уровень убедительности рекомендаций А, уровень достоверности доказательств 1 ++) [20] Imiquimod (recommendation level A, level of evidence 1++) [20]
Кюретаж и электрокоагуляция (уровень убедительности рекомендаций D, уровень достоверности доказательств 4) Curettage and electrodesication (grade of recommendation D, level of evidence 4)	Мебутат ингенола (уровень убедительности рекомендаций А, уровень достоверности доказательств 1+) [20] Ingenol mebutate (recommendation level A, evidence level 1+) [20]
Хирургическое лечение (уровень убедительности рекомендаций D, уровень достоверности доказательств 4) Surgical exeresis (grade of recommendation D, level of evidence 4)	Фотодинамическая терапия (уровень убедительности рекомендаций А, уровень достоверности доказательств 1+) [20] Photodynamic therapy (recommendation grade A, evidence level 1+) [20]
	Диклофенак (уровень убедительности рекомендаций А, уровень достоверности доказательств 1+) [20] Diclofenac (recommendation level A, evidence level 1+) [20]
	Ретиноиды для местного применения (уровень убедительности рекомендаций В, уровень достоверности доказательств 1+) [20] Topical retinoids (recommendation level B, evidence level 1+) [20]
	Системная терапия (уровень убедительности рекомендаций С, уровень достоверности доказательств 2+) [20] Systemic therapy (recommendation grade C, level of evidence 2+) [20]

Increasingly, combinations of several methods are being considered, for example, after treating a local focus, the treatment of the tumor field is resorted to. Before PDT, laser ablation can be performed to remove foci of volumetric hyperkeratotic lesions, thereby making the skin surface more permeable for applying the cream. Other studies have shown that the combination of imiquimod and PDT results in significantly higher healing rates than imiquimod monotherapy [24, 25].

Operative therapy

Curettage

The use of curettage under local anesthesia can be performed alone or in combination with electrocoagulation, which appears to increase devitalization of potentially remaining dysplastic cells, and to achieve hemostasis. An alternative to electrocoagulation is cryotherapy [19]. As monotherapy, curettage is especially indicated in patients with solitary lesions, especially those with hyperkeratotic AK. The lack of randomized clinical trials to evaluate the effectiveness of the method leads to a low degree of recommendation of the procedure for the treatment of AK [26].

Cryotherapy

Cryotherapy is a destructive method used for the isolated treatment of AK, which uses liquid nitrogen to achieve the processes of freezing and thawing of tissues leading to their destruction [26]. Cryotherapy is indicated for the treatment of patients with single or small lesions without tumor fields. Despite widespread use in dermatological practice, persistent complete remission after cryotherapy in patients with isolated lesions at 12 months of follow-up is lower (28%) than in patients treated with 5-FU (54%) and imiquimod (73%). This is precisely because some patients have preclinical changes in the immediate vicinity of the treated lesions [22]. The advantages of this treatment method are low cost, easy accessibility and satisfactory patient compliance. The disadvantages include the fact that the method does not allow to treat tumor fields, causes discomfort during application and has a long rehabilitation time. Cryotherapy has received a sufficient level of persuasiveness of recommendations for the treatment of localized lesions in patients without immunosuppression, while at the same time in patients with immunosuppression, the effect is limited [26].

CO_2 laser

Lasers cause coagulative necrosis, ablation and hyperthermia, which leads to the destruction of the lesion. One session of non-fractional CO_2 laser can be used to remove superficial lesions of the epidermis, including AK. A non-fractional CO_2 laser with a wavelength of 10,600 nm is absorbed by water, resulting in non-specific tissue destruction. Thus, a non-fractional CO_2 laser can be used to destroy localized lesions. The results of the full therapeutic effect in the first months are similar to those obtained with cryotherapy (72.8% in the laser group versus 78% in the cryotherapy group). However, on long-term follow-up, lesions treated with CO_2 laser have a lower objective response rate: no recurrence was found in 37% of patients treated with laser compared to 66.8% of patients treated with cryotherapy [27]. Due to the increased risk of infection in immunosuppressed patients, CO_2 laser is not recommended for the treatment of tumor fields and should only be used for localized lesions [26]. Although the use of CO_2 laser can be considered as a treatment option for AK, the degree of recommendation for its use in patients with a normal immune response is not conclusive [28].

Non-surgical treatment

5-fluorouracil

5-Fluorouracil (5-FU) is used in AK at concentrations ranging from 0.5% to 5%. 5-FU prevents DNA synthesis due to irreversible inactivation of thymidylate synthase; the end result is apoptosis of highly proliferating cells such as keratinocytes in AK lesions [29].

Imichimod

Imichimod is a synthetic compound from the imidazoquinoline family that acts as an immunomodulator. The drug acts as an instrumental receptor in the expression of mRNA of immunomodulatory genes that induce the production of cytokines; as a result, the innate and acquired immune response with increased antitumor and antiviral activity is stimulated [30].

The strength of recommendation for the treatment of tumor fields with imichimod 3.75% is high, while for imichimod 5% the strength of recommendation is low; this difference is due to the methodological quality of the studies [26].

Ingenol mebutate

Ingenol mebutate (IM) is derived from the plant Euphorbia peplus. Recommended for the treatment of AK of the scalp and face at a concentration of 0.015% for three consecutive days (one vial per day); for use outside the face at a concentration of 0.05% for two consecutive days. IM has two mechanisms of action mediated by neutrophils - cytotoxic and immunomodulatory [31].

Diclofenac

The use of 3% diclofenac gel in combination with 2.5% hyaluronic acid, used to optimize the penetration of diclofenac into the epidermis, is recommended for the treatment of AK twice a day for a minimum period of 60–90 days [32]. The therapy action mechanism is the inhibition of cyclooxygenase-2 (COX-2), which leads to a decrease in prostaglandin synthesis and inhibition of cell differentiation and angiogenesis, induction of apoptosis and changes in cell proliferative activity [32]. Diclofenac also activates hormone receptors in the cell nucleus involved in cell differentiation and apoptosis [32].

Photodynamic therapy

The PDT technique consists of using a photosensitizing agent (PS) and a light source of a certain wavelength to generate reactive oxygen species, which then destroy dysplastic cells through a photochemical reaction [33]. This reaction is achieved by the use of 5-ALA or MAL, which are precursors to the photoactive metabolites of PPIX. These metabolites accumulate in neoplastic cells and, when activated by visible light, lead to the formation of singlet oxygen [33]. They initiate a biochemical cascade of events that cause death of the target cell as a result of apoptosis or necrosis and immunomodulatory action [34]. The photochemical reaction for each PS occurs after irradiation with a light source of a certain wavelength in the visible light spectrum [35]. The spectrum has four porphyrin absorption peaks, the largest of which is in the blue light spectrum at 410 nm, with smaller peaks at 540 nm, 580 nm, and 635 nm [36]. Red light (625–740 nm) penetrates deeper than blue light and is therefore preferred in the treatment of larger lesions. The blue light spectrum (440–485 nm) due to absorption by hemoglobin reaches a depth of 1–2 mm and is commonly used to treat superficial lesions [36]. Light emitting diode (LED) devices are the most commonly used light sources for PDT and are considered the gold standard [37]. Superficial curettage of lesions is recommended prior to application of PS to the treated area [36]. In addition, techniques that increase the depth of PS penetration can be used: microneedling, ablative fractional laser, the use of calcipotriol [36, 38]. According to the Cochrane review of AK treatment, PDT with 5-ALA or MAL using both red and blue LED light has similar efficacy [22].

PDT with 5-ALA was approved by the FDA for AK therapy back in 1999 [39]. Since then, it has been widely used for the treatment of AK foci and tumor fields with the same efficacy demonstrated in controlled clinical trials [40, 41]. In 2016, a clinical consensus guideline stated that PDT for AK is highly effective for head and neck lesions and is similar or superior to other FDA-approved therapies. The cosmetic results of PDT are superior to those of cryotherapy [42]. New recommendations for the treatment of AK from the American Academy of Dermatology (AAD) were released in 2021 [43]. The International League of Dermatological Societies, in collaboration with the European Dermatology Forum, recommend PDT for patients with tumor fields [44]. The British Association of Dermatologists guidelines for the treatment of patients with AK state that PDT is an effective treatment for confluent AK lesions in the absence of invasive disease. Otherwise, confluent AK lesions in areas such as the scalp are difficult to treat or not treatable at all. The British Association of Dermatologists also notes that PDT has a low potential for scarring and reduces the risk of poor healing compared to other therapies in certain areas, such as the lower leg [45]. The British Association of Dermatologists has provided guidance on the use of PDT for the treatment of AK, focusing on drugs and light sources, and recommends this treatment (level A recommendation, quality of evidence 1) [46].

In the conventional MAL PDT protocol, a thin layer of product 1 mm thick should be applied to the treated area, which is closed for 3 hours, then the area should be cleaned and irradiated with the selected light source. The main randomized controlled trials evaluating the complete response rate 3 months after PDT with ALA showed that 69-91% of treated patients achieved complete resolution of the lesions. In a meta-analysis that included 641 participants with 2,174 cryo-treated AK lesions and 2,170 PDT-treated AK lesions, participants achieved 14% more complete responses in the PDT group. Complete remission with PDT with MAL after 3 months was achieved in 90% of cases [47], excellent cosmetic results were noted in 91-98% of treated patients [48].

The results of controlled clinical trials also confirm the effectiveness of PDT as the main therapy for patients with AK [49]. An analysis of comparative clinical studies has also shown that PDT is no less effective than other approaches to tumor field therapy, including imiquimod [50], chemical skin peels [51], diclofenac [32] and 5-FU [52]. A clinical study in Europe evaluated the efficacy of 5-FU (4–8 weeks), imiquimod (4–8 weeks), ingenol mebutate (3–6 days), and MAL-PDT (1–2 sessions). It was found that 12 months after completion of treatment, the probability of avoiding therapy failure (clearance > 75%) was significantly higher in patients treated with 5-FU compared with other compared methods [53]. PDT has also demonstrated rejuvenating effects in actinic degeneration and other signs of aging from sun damage [54].

PDT has a high level of persuasiveness of recommendations for the treatment of AKs and tumor fields [44], so the method is most suitable for patients with multiple AKs. Generally, the benefits of PDT include few long-term side effects, reproducible outpatient efficacy, non-invasiveness of the procedure, patient compliance, and the ability to address subclinical lesions. Potential risks include increased skin sensitivity to light for 24 to 48 hours after treatment and possible side effects at the PDT site for about two weeks, including short-term skin swelling, sloughing, scabs, blisters, itching, burning, and (rarely) skin infections. [55]. About 20% of patients complain of severe pain (pain grade over 6 on a scale of 0 to 10) during LED irradiation and remain with intense erythema and scaling for up to 21 days [56]. Physicians should consider possible contraindications to PDT before prescribing treatment, including hypersensitivity to porphyrins or

any component of the 5-ALA gel (often 10%), porphyria, or photodermatoses. Before starting PDT, a physical examination should be performed to assess the presence of skin cancer in the proposed treatment area. In addition to grade I–III AK, PDT can treat patients with morphologically confirmed in situ SCSC or superficial BCSC. Lesions suspected of melanoma, invasive SCSC, or BCSC should be biopsied and treated with other methods. The presence or absence of a history of herpes simplex should be confirmed, and patients with a positive history should be given valaciclovir or famciclovir prior to PDT. The use of PDT is also limited in pigmented lesions, which reduce the efficiency of the photochemical reaction, since the melanin pigment competes with PPIX in absorbing light, reducing the desired photodynamic effect [57].

In recent years, daylight PDT has been described as having the same response rate as classical PDT, but with fewer radiation-related side effects [58]. The PDT method using daylight consists in applying MAL cream to the focus, then, after 30 min, placing the patient in daylight for about 2 hours in order to activate MAL with visible light in the wavelength range from 380 to 740 nm [59]. Daylight PDT is mainly recommended for the treatment of non-pigmented AKs [60]. Studies comparing conventional PDT and daylight PDT have demonstrated the same efficacy and safety of both AK treatments on the face and scalp [61]. Both 5-ALA PDT and MAL PDT are highly recommended for the treatment of multiple lesions and tumor fields.

PDT was effectively combined with other topical drugs in the treatment of tumor fields [62, 63]. A meta-analysis including the results of 10 randomized controlled trials in which PDT was combined with imiquimod, 5-FU, ingenol mebutate, tazarotene, or calcipotriol showed that the use of a combination of methods improved overall response rates compared with the separate use of PDT or topical agents [24].

PDT has also been used to prevent AK and NMSC in organ transplant recipients. In a small pilot study, 12 high-risk patients received cyclic 5-ALA-PDT at intervals of 4 to 8 weeks for two years. The median reduction in the incidence of SCSC after 12 and 24 months was 79% and 95%, respectively [64]. Repeated use of PDT was a primary method of skin dysplasia prevention in kidney transplant recipients. In a randomized trial involving 25 patients with clinically normal skin who received MAL-PDT at 6-month intervals for five years, a 63% reduction in the formation of new AKs in previously treated skin areas was observed [65]. Sequential daylight PDT has been shown to be effective in preventing the appearance of new AK and NMSC lesions in organ transplant patients. The treatment of tumor fields was also accompanied by a significantly lower appearance of new lesions in dynamics, higher adherence of patients to treatment compared with the control group, which used cryotherapy [66]. A systematic review published in 2020 and a meta-analysis of 12 studies involving organ transplant patients favored the use of PDT as an effective preventive measure for the development of AK and SCSC [67].

Conclusion

One of the problems of AK treatment is a high percentage of relapses in tumor fields. Therefore, treatment should cover not only AK foci, but also visually unchanged tissues, which limits the use of surgical techniques due to the volume of intervention and the severity of the surgical injury, which affect the cosmetic results of treatment and the duration of rehabilitation.

If the patient has tumor fields, it is necessary to consider the possibilities of other methods of therapy, that allow, with satisfactory tolerability, to carry out treatment on a wide area of AK lesion. However, as systematic reviews have shown, the better the tolerability of a particular non-surgical method, the lower its effectiveness [68, 69].

If it is necessary to treat large tumor fields, PDT can become an ideal method of treatment, during which it is possible to achieve both high therapeutic efficacy - up to 90% complete regressions, and good cosmetic results in 91-98% of patients [57, 66].

The main side effect of PDT is pain during treatment, which is described as a burning and tingling sensation localized to the area of treatment. Currently, research is being carried out aimed at modifying PDT techniques to achieve better treatment tolerance without reducing its effectiveness. The European-approved PDT technology with daylight activation is as effective as the classical version, but is better tolerated and almost painless [61]. Unfortunately, daylight-activated PDT is weather dependent and cannot be performed in rainy, windy, or cold weather [70]. Moreover, due to the varying intensity of daylight depending on weather conditions and location, it is not possible to control the light dose. New PDT protocols, including the Flexitheralight protocol [71], can significantly reduce the level of pain when using PDT technology with daylight activation without loss of efficiency, expand the conditions for its use: regardless of the season and weather conditions, with a known dose of light, corresponding in this respect to the classical PDT protocol [33].

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