ВНУТРЕННИЕ БОЛЕЗНИ

INTERNAL MEDICINE

DOI: 10.12731/2658-6649-2022-14-4-11-22
UDC 616-006.04

NEW PROMISING AGENTS FOR PHOTODYNAMIC THERAPY

O.V. Shevchenko, N.G. Plekhova, O.V. Korshunova, I.G. Tananaev, V.I. Apanasevich

The problem of oncological diseases has acquired a social and medical character. In this regard, the rapid development of new drugs and methods, as well as modification of existing methods in order to increase their effectiveness, is currently required. The paper aims to develop and study two promising agents for photodynamic therapy. We discussed a new binary complex based on the widely used photosensitizer chlorin E6 with europium, which can solve one of the main disadvantages of drugs for photodynamic therapy: it can be used to treat deeper tumors and act more effectively by transferring luminescence energy between europium and chlorin E6. Our other agent was copper-containing chlorophyllin. Chlorophyllin is a natural component, which is always promising in the development of new drugs. The optical and fluorescent parameters of the agents were determined using physicochemical methods; their ability to generate reactive oxygen species in the presence of two fluorescent probes was studied: 2', 7'-dichlorodihydrofluorescein and dihydrofluorescein. Thus, we showed the effectiveness of chlorophyllin in comparison with chlorin E6 by 1.92 times at 50 μg / ml E6 and showed the effectiveness of the binary complex even at the minimum concentration of 10 μg / ml. Further research in cell cultures will develop the ways of the possible use of new substances as photosensitizers.

Keywords: *chlorin E6; chlorophyllin; binary complex; Europium; red spectrum; reactive oxygen species; photodynamic therapy; oncology*

For citation. *Shevchenko O.V., Plekhova N.G., Korshunova O.V., Tananaev I.G., Apanasevich V.I. New Promising Agents for Photodynamic Therapy. Siberian Journal of Life Sciences and Agriculture, 2022, vol. 14, no. 4, pp. 11-22. DOI: 10.12731/2658-6649-2022-14-4-11-22*

НОВЫЕ ПЕРСПЕКТИВНЫЕ АГЕНТЫ ДЛЯ ФОТОДИНАМИЧЕСКОЙ ТЕРАПИИ

О.В. Шевченко, Н.Г. Плехова, О.В. Коршунова, И.Г. Тананаев, В.И. Апанасевич

Проблема онкологических заболеваний приобрела социальный и медицинский характер. В связи с этим в настоящее время требуется быстрая разработка новых препаратов и методов, а также модификация существующих методов с целью повышения их эффективности. Данная работа направлена на разработку и изучение двух перспективных агентов для фотодинамической терапии. Мы рассмотрели новый бинарный комплекс на основе широко используемого фотосенсибилизатора хлорина Е6 с европием, который может решить один из основных недостатков препаратов для фотодинамической терапии: его можно использовать для лечения более глубоких опухолей и действовать более эффективно за счет передачи люминесценции между европием и хлорином Е6. Другим нашим агентом был медьсодержащий хлорофиллин. Хлорофиллин – это природный компонент, который всегда является перспективным при разработке новых лекарств. Оптические и флуоресцентные параметры агентов были определены с использованием физико-химических методов; была изучена их способность генерировать активные формы кислорода в присутствии двух флуоресцентных зондов: 2', 7'-дихлордигидрофлуоресцеина и дигидрофлуоресцеина. Таким образом, мы показали эффективность (1) хлорофиллина по сравнению с хлорином Е6 в 1,92 раза при 50 мкг/мл Е6 и (2) бинарного комплекса даже при минимальной концентрации 10 мкг/мл. Дальнейшие исследования на клеточных культурах позволят разработать пути возможного использования новых веществ в качестве фотосенсибилизаторов.

Ключевые слова: *хлорин Е6; хлорофиллин; бинарный комплекс; Европий; красный спектр; активные формы кислорода; фотодинамическая терапия; онкология*

Для цитирования. Шевченко О.В., Плехова Н.Г., Коршунова О.В., Тананаев И.Г., Апанасевич В.И. Новые перспективные агенты для фотодинамической терапии // Siberian Journal of Life Sciences and Agriculture. 2022. Т. 14, №4. С. 11-22. DOI: 10.12731/2658-6649-2022-14-4-11-22

Introduction

Scientists predict that by 2040, the number of newly diagnosed cancers will increase by 1.5 times compared to 2020 and will amount to 26 million people [12]. The method of photodynamic therapy [PDT] is currently being widely

developed due to the expansion of scientific knowledge, the capabilities of the instrumental base, as well as the rapidly increasing statistics of cancer.

Medicinal products of natural origin are beginning to occupy a leading position in many countries. Historically, the first photodynamic agents were natural ingredients [8]. The photosensitizer [PS] chlorophyllin is a derivative of the known chlorophyll compound. The main structure of chlorophyll is the porphyrin ring, which is analogous to the structure of heme in hemoglobin. The phytol tail of the porphyrin ring makes it fat-soluble and insoluble in water. Chlorophyllin is a semi-synthetic mixture of sodium-copper salts derived from chlorophyll [6]. During chlorophyllin synthesis, the magnesium atom in the center of the ring is replaced with copper, and the phytol tail is lost. Unlike natural chlorophyll, chlorophyllin is soluble in water, which is one of the essential requirements for a photosensitizer [7]. Currently, the chlorin E6 preparation synthesized in the early 90s in the Russian Federation from cyanobacteria is known [3]; it is widely used in many countries, as well as modified in different ways to enhance the effectiveness of photodynamic therapy [1].

In our work, we study the prospect of using the synthesized binary complex of chlorin E6 with europium not only for photodynamics but also for radiotherapy due to the introduction of a heavy element into the structure. Europium is capable of luminescence when exposed to coarse radiation (gamma or bremsstrahlung), as well as the transfer of this energy to the photosensitizer chlorin E6. We assume that in this way, it is possible to implement more effective therapy, for example, the treatment of deep tumors.

Materials and Methods

Thus, the paper aims to study the possibility of a new generation of photosensitizers (chlorophyllin and a binary complex based on chlorin E6) to act as promising agents for photodynamic therapy.

At this research stage, the objectives are as follows:

- Synthesize a stable binary complex based on chlorin E6 with europium and select the optimal conditions for its further preparation.
- Investigate the optical and fluorescent parameters of the binary complex and copper-containing chlorophyllin using physicochemical research methods.
- Investigate the possibility of generating reactive oxygen species by two possible photodynamic agents in the presence of fluorescent probes.

To fulfill the set goal and objectives, several conditions were used.

The binary complex of chlorin E6 with europium was obtained at pH 6.5 by mixing a solution of chlorin E6 (OOO Veta-Grand, Russia, concentrate 5 mg

/ ml) and EuCl3 (Sigma-Aldrich, USA, 100 mg / ml) in a molar ratio of 11.7 mmol / 2, 93 mmol, respectively. The synthesis was carried out in the absence of light with stirring on a magnetic stirrer for 6 hours. The resulting solutions were dialyzed to remove low molecular weight compounds and excess europium against distilled water for 3 days and freeze-dried. Chlorophyllin from alfalfa was obtained from a company (Sigma-Aldrich, USA).

Absorption spectra were obtained in the range of 300–700 nm on spectrofluorimeters Synergy H1 (BioTek, USA) and RF-5301 PC (Shimadzu, Japan) in phosphate-buffered saline (PBS) at pH 7.4.

Irradiation with a red spectrum was carried out through a semiconductor laser (Light and Life, Tomsk, Russia) with an output power of 32 mW at a light wavelength of 645 nm, giving an irradiation dose of 6 J/cm^2.

The generation of reactive oxygen species [ROS] was noted in the presence of fluorescent probes 2′,7′-dichlorodihydrofluorescein (Sigma-Aldrich, USA) or dihydrofluorescein (Sigma-Aldrich, USA) on Synergy H1 (BioTek, USA) under optimal conditions ex / em 485/530.

Results

The obtained absorption spectra demonstrate the presence of characteristic absorption maxima, analogous to chlorin E6, which is part of the structures of chlorophyllin and the binary complex (Fig. 1). This fact is fully consistent with the data presented in the literature [2].

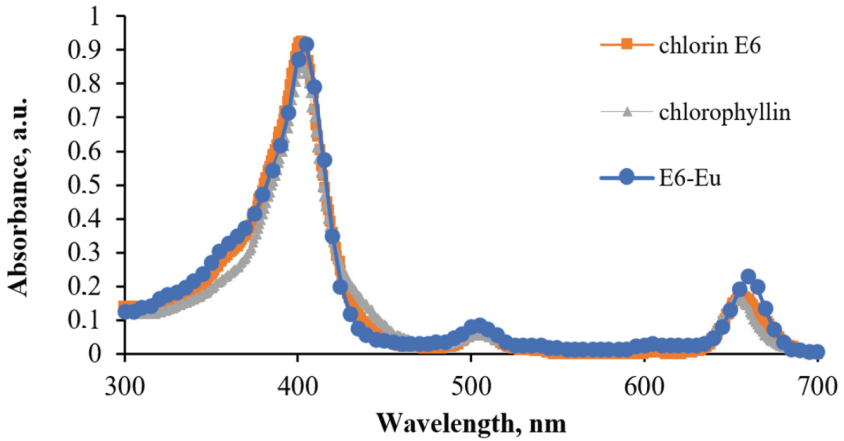

Fig. 1. Absorption spectra of E6-Eu, chlorin E6 and chlorophyllin at 50 μg/ml E6; $p<0,05$.

The E6 content was calculated at a characteristic absorption maximum of 400 nm since this wavelength is functionally significant during therapy [4]. This fact is important since modern LEDs with selective wavelength are used for photodynamic therapy.

In addition to optical fibers, dye lasers (600–700 nm), quantum scopes, semiconductor lasers (400–1270 nm), and various non-laser light sources are widely used [5].

Irradiation with the red spectrum of 645 nm was carried out for 30 min with an interval of 5 min in the presence of 4 μg/ml fluorescent probe 2′, 7′-dichlorodihydrofluorescein (Fig. 2). A significant increase in fluorescence has been shown for chlorophyllin compared to chlorin E6. In addition, the increase in fluorescence for a binary complex in 5 min is 1.5–2 times more intense.

Fig. 3 shows the generation of reactive oxygen species for chlorophyllin and chlorin E6 at an E6 content of 50 μg/ml. At this concentration, the effectiveness of chlorophyllin at 30-min irradiation is 1.92 times.

Similar studies were conducted for the synthesized of binary conjugate under irradiation for 60 minutes. In this case, dihydrofluorescein was used as a fluorescent probe. Fig. 4 shows the experimental data at an E6 content of 10 μg/ml for both photosensitizers.

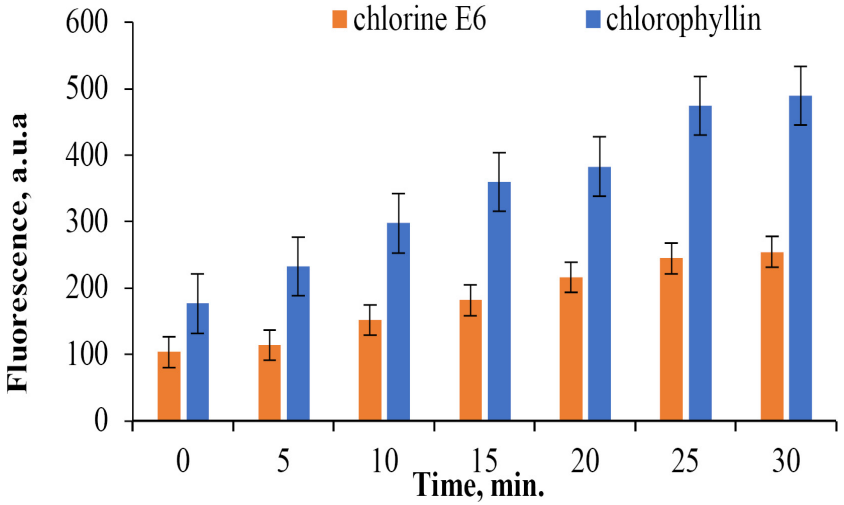

Fig. 2. An increase in fluorescence upon irradiation with red light in chlorin E6 and chlorophyllin at 80 μg/ml E6 for 30 minutes; $p<0,05$.

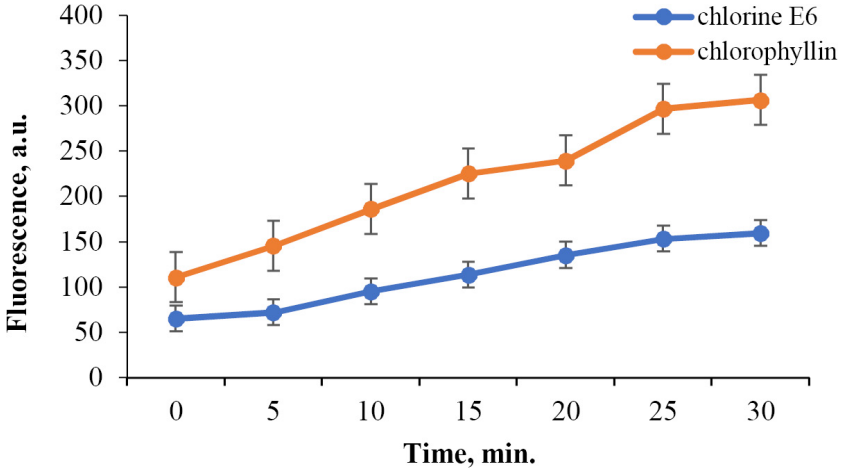

Fig. 3. Generation of reactive oxygen species by the test substances at 50 μg/ml E6 in the presence of a fluorescent probe; $p<0,05$.

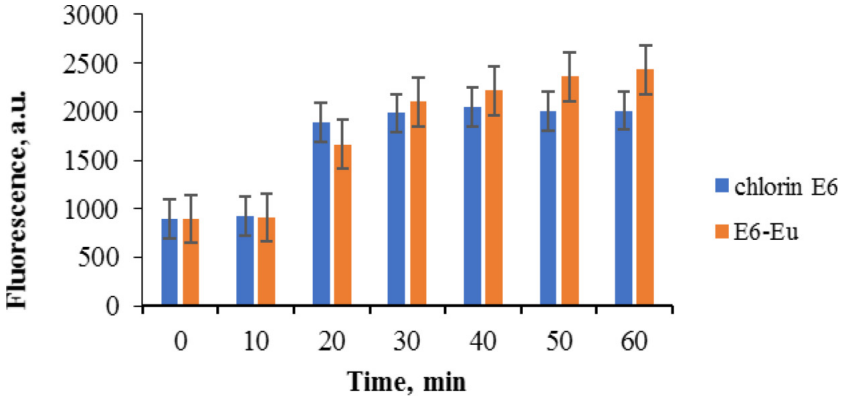

Fig. 4. Increase in fluorescence upon irradiation with a red light in chlorin E6 and E6-Eu for 60 min.

Discussion

As mentioned earlier, one of the limitations of the photodynamic therapy method is the penetration depth of laser radiation into biological tissue. The drugs used in clinical practice are excited by light in the 620–690 nm region [11]. The permeability of biological tissues in this range is insignificant: only a few millimeters. The maximum tissue permeability is in the far red and near

infrared regions of the spectrum (750–1500 nm). That is, the absorption maxima of the main PS go beyond the tissue optical window (700–1100 nm), where most tissue chromophores, including hydroxy and deoxyhemoglobin, melanin, and fat have only weak absorption. The scholars [9] obtained hypericin derivatives. Hypericin has excellent photosensitizing properties and exhibits anti-tumor activity as well as minimal toxicity in the dark [10]. Potentially derived structures have an advantage over the parent compound due to photoactivation by red light, which penetrates deeper into the tumor tissue. The results show that three compounds (dibenzoxazole, pyridazinone and especially the dibenzthiazole derivative hypericin), capable of a bathochromic shift in the absorption spectrum, demonstrated efficient intracellular absorption and generation of singlet oxygen. In addition, they have a potent photocytotoxic effect under white light conditions.

However, the data also show that the present derivatives are unproductive photosensitizers when used under red light conditions, showing little generation of reactive oxygen species.

A binary complex synthesized by us as a PDT agent was also considered; it is worth noting its effectiveness in relation to the generation of ROS even at minimal concentrations. The copper-containing chlorophyllin and binary complex studied by us manifest themselves at the initial stages of the study as promising agents for PDT, the effectiveness of which exceeds the widely used chlorin E6.

Conclusion

The heavy element europium introduced into the structures of the complex makes it possible to develop a combined method of radiophotodynamic therapy of deep malignant neoplasms. The data obtained confirm the relevance and perspective of further photodynamic studies of chlorophyllin and the binary complex.

Acknowledgments. The reported study was funded by Russian Foundation for Basic Research, project No. 20-33-90185.

References

1. Abrahamse H., Hamblin, M. R. New photosensitizers for photodynamic therapy. *Biochemical Journal*, 2016, vol. 473, no. 4, pp. 347-364. URL: https://pubmed.ncbi.nlm.nih.gov/26862179/
2. Bharathiraja S., Moorthy M. S., Manivasagan P., Seo H., Lee K. D., Oh, J. Chlorin e6 conjugated silica nanoparticles for targeted and effective photodynamic

therapy. *Photodiagnosis and Photodynamic Therapy*, 2017, vol. 19, pp. 212-220. https://doi.org/10.1016/j.pdpdt.2017.06.001

3. Dadeko A. V., Muravyova T. D., Starodubtsev A. M., Belousova I. M. Study of the photophysical properties of a water-soluble photosensitizer of porphyrin nature—dimegin. *Journal of Optical Technology*, 2016, vol. 83, no. 3, pp. 193-196. https://doi.org/10.1364/JOT.83.000193
4. Khilov A. V., Kirillin M. Y., Loginova D. A., Turchin, I. V. Estimation of chlorin-based photosensitizer penetration depth prior to photodynamic therapy procedure with dual-wavelength fluorescence imaging. *Laser Physics Letters*, 2018, vol. 15, no. 12, e126202.
5. Kim M. M., Darafsheh A. Light sources and dosimetry techniques for photodynamic therapy. *Photochemistry and Photobiology*, 2020, vol. 96, no. 2, pp. 280-294. URL: https://pubmed.ncbi.nlm.nih.gov/32003006/
6. Luksiene Z., Buchovec I. Impact of chlorophyllin-chitosan coating and visible light on the microbial contamination, shelf life, nutritional and visual quality of strawberries. *Innovative Food Science & Emerging Technologies*, 2019, vol. 52, pp. 463-472. https://doi.org/10.1016/j.ifset.2019.02.003
7. Mokwena M. G., Kruger C. A., Ivan M. T., Heidi A. A review of nanoparticle photosensitizer drug delivery uptake systems for photodynamic treatment of lung cancer. *Photodiagnosis and Photodynamic Therapy*, 2018, vol. 22, pp. 147-154. https://doi.org/10.1016/j.pdpdt.2018.03.006
8. Polat E., Kang K. Natural photosensitizers in antimicrobial photodynamic therapy. *Biomedicines*, 2021, vol. 9, no. 6, pp. 584. https://doi.org/10.3390/biomedicines9060584
9. Roelants M., Lackner B., Waser M., Falk H., Agostinis P., Van Poppel H., De Witte P. A. In vitro study of the photocytotoxicity of bathochromically-shifted hypericin derivatives. *Photochemical & Photobiological Sciences*, 2009, vol. 8, no. 6, pp. 822-829. URL: https://pubs.rsc.org/en/content/articlelanding/2009/PP/b820817d
10. Vandenbogaerde A. L., Kamuhabwa A., Delaey E., Himpens B. E., Merlevede W. J., de Witte P. A. Photocytotoxic effect of pseudohypericin versus hypericin. *Journal of Photochemistry and Photobiology B: Biology*, 1998, vol. 45, no. 2-3, pp. 87-94. https://doi.org/10.1016/S1011-1344(98)00163-8
11. Vera J., Bayazitoglu Y. A note on laser penetration in nanoshell deposited tissue. *International Journal of Heat and Mass Transfer*, 2009, vol. 52, no. 13-14, pp. 3402-3406. https://doi.org/10.1016/j.ijheatmasstransfer.2009.02.014
12. Wilson B. E., Jacob S., Yap M. L., Ferlay J., Bray F., Barton M. B. Estimates of global chemotherapy demands and corresponding physician workforce require-

ments for 2018 and 2040: a population-based study. *The Lancet Oncology*, 2019, vol. 20, no. 6, pp. 769-780. https://doi.org/10.1016/S1470-2045(19)30375-4

Список литературы

1. Abrahamse H., Hamblin, M. R. New photosensitizers for photodynamic therapy // Biochemical Journal, 2016, vol. 473, no. 4, pp. 347-364. URL: https://pubmed.ncbi.nlm.nih.gov/26862179/
2. Bharathiraja S., Moorthy M. S., Manivasagan P., Seo H., Lee K. D., Oh, J. Chlorin e6 conjugated silica nanoparticles for targeted and effective photodynamic therapy // Photodiagnosis and Photodynamic Therapy, 2017, vol. 19, pp. 212-220. https://doi.org/10.1016/j.pdpdt.2017.06.001
3. Dadeko A. V., Muravyova T. D., Starodubtsev A. M., Belousova I. M. Study of the photophysical properties of a water-soluble photosensitizer of porphyrin nature—dimegin // Journal of Optical Technology, 2016, vol. 83, no. 3, pp. 193-196. https://doi.org/10.1364/JOT.83.000193
4. Khilov A. V., Kirillin M. Y., Loginova D. A., Turchin, I. V. Estimation of chlorin-based photosensitizer penetration depth prior to photodynamic therapy procedure with dual-wavelength fluorescence imaging // Laser Physics Letters, 2018, vol. 15, no. 12, e126202.
5. Kim M. M., Darafsheh A. Light sources and dosimetry techniques for photodynamic therapy // Photochemistry and photobiology, 2020, vol. 96, no. 2, pp. 280-294. URL: https://pubmed.ncbi.nlm.nih.gov/32003006/
6. Luksiene Z., Buchovec I. Impact of chlorophyllin-chitosan coating and visible light on the microbial contamination, shelf life, nutritional and visual quality of strawberries // Innovative Food Science & Emerging Technologies, 2019, vol. 52, pp. 463-472. https://doi.org/10.1016/j.ifset.2019.02.003
7. Mokwena M. G., Kruger C. A., Ivan M. T., Heidi A. A review of nanoparticle photosensitizer drug delivery uptake systems for photodynamic treatment of lung cancer // Photodiagnosis and Photodynamic Therapy, 2018, vol. 22, pp. 147-154. https://doi.org/10.1016/j.pdpdt.2018.03.006
8. Polat E., Kang K. Natural photosensitizers in antimicrobial photodynamic therapy // Biomedicines, 2021, vol. 9, no. 6, pp. 584. https://doi.org/10.3390/biomedicines9060584
9. Roelants M., Lackner B., Waser M., Falk H., Agostinis P., Van Poppel H., De Witte P. A. In vitro study of the photocytotoxicity of bathochromically-shifted hypericin derivatives // Photochemical & Photobiological Sciences, 2009, vol. 8, no. 6, pp. 822-829. URL: https://pubs.rsc.org/en/content/articlelanding/2009/PP/b820817d

10. Vandenbogaerde A. L., Kamuhabwa A., Delaey E., Himpens B. E., Merlevede W. J., de Witte P. A. Photocytotoxic effect of pseudohypericin versus hypericin // Journal of Photochemistry and Photobiology B: Biology, 1998, vol. 45, no. 2-3, pp. 87-94. https://doi.org/10.1016/S1011-1344(98)00163-8
11. Vera J., Bayazitoglu Y. A note on laser penetration in nanoshell deposited tissue // International journal of heat and mass transfer, 2009, vol. 52, no. 13-14, pp. 3402-3406. https://doi.org/10.1016/j.ijheatmasstransfer.2009.02.014
12. Wilson B. E., Jacob S., Yap M. L., Ferlay J., Bray F., Barton M. B. Estimates of global chemotherapy demands and corresponding physician workforce requirements for 2018 and 2040: a population-based study // The Lancet Oncology, 2019, vol. 20, no. 6, pp. 769-780. https://doi.org/10.1016/S1470-2045(19)30375-4

ВКЛАД АВТОРОВ

Все авторы сделали эквивалентный вклад в подготовку статьи для публикации.

AUTHOR CONTRIBUTIONS

The authors contributed equally to this article.

DATA ABOUT THE AUTHORS

Olga V. Shevchenko

Pacific State Medical University, Ministry of Health of Russia; Far Eastern Federal University
2, Ostryakov Ave., Vladivostok, 690002, Russian Federation; 8, Sukhanova Str., Vladivostok, 690091, Russian Federation
tarakovaolga@gmail.com
ORCID: https://orcid.org/0000-0002-3113-3995

Natalya G. Plekhova

Pacific State Medical University, Ministry of Health of Russia
2, Ostryakov Ave., Vladivostok, 690002, Russian Federation
pl_nat@hotmail.com
ORCID: https://orcid.org/0000-0002-8701-7213

Oksana V. Korshunova

Pacific State Medical University, Ministry of Health of Russia
2, Ostryakov Ave., Vladivostok, 690002, Russian Federation

farmaoks@yandex.ru
ORCID: https://orcid.org/0000-0003-3533-8506

Ivan G. Tananaev
Far Eastern Federal University
8, Sukhanova Str., Vladivostok, 690091, Russian Federation
geokhi@mail.ru
ORCID: https://orcid.org/0000-0002-2159-8182

Vladimir I. Apanasevich
Pacific State Medical University, Ministry of Health of Russia
2, Ostryakov Ave., Vladivostok, 690002, Russian Federation
oncolog222@gmail.com
ORCID: https://orcid.org/0000-0003-0808-5283

ДАННЫЕ ОБ АВТОРАХ

Шевченко Ольга В.
Тихоокеанский государственный медицинский университет Минздрава России; Дальневосточный федеральный университет
пр. Острякова, 2, г. Владивосток, 690002, Российская Федерация; ул. Суханова, 8, г. Владивосток, 690091, Российская Федерация
tarakovaolga@gmail.com

Плехова Наталья Г.
Тихоокеанский государственный медицинский университет Минздрава России
пр. Острякова, 2, г. Владивосток, 690002, Российская Федерация
pl_nat@hotmail.com

Коршунова Оксана В.
Тихоокеанский государственный медицинский университет Минздрава России
пр. Острякова, 2, г. Владивосток, 690002, Российская Федерация
farmaoks@yandex.ru

Тананаев Иван Г.
Дальневосточный федеральный университет
ул. Суханова, 8, г. Владивосток, 690091, Российская Федерация
geokhi@mail.ru

Апанасевич Владимир И.

Тихоокеанский государственный медицинский университет Минздрава России
пр. Острякова, 2, г. Владивосток, 690002, Российская Федерация
oncolog222@gmail.com

Поступила 29.03.2022
После рецензирования 25.04.2022
Принята 30.04.2022

Received 29.03.2022
Revised 25.04.2022
Accepted 30.04.2022