DOI: 10.6060/ivkkt.20236612.6902

УДК: 547-97; 551.464.797.9; 615-28

ПРИРОДНЫЕ ХЛОРИНОВЫЕ ФОТОСЕНСИБИЛИЗАТОРЫ И ПОТЕНЦИРУЮЩИЕ АГЕНТЫ ДЛЯ АНТИМИКРОБНОЙ ФОТОДИНАМИЧЕСКОЙ ТЕРАПИИ

А.В. Кустов

Андрей Владимирович Кустов (ORCID 0000-0002-3553-6206)

Институт химии растворов им. Г.А. Крестова РАН, Академическая ул., 1, Иваново, Российская Федерация, 153045
E-mail: kustov@isuct.ru

Данные всемирной организации здравоохранения свидетельствуют, что эра антибиотиков постепенно подходит к концу, и вероятность открытия новых классов антимикробных препаратов оценивается как низкая, необходимо найти альтернативные технологии для борьбы с антибиотикорезистентными микроорганизмами. Ключевой вопрос, на который необходимо ответить в последние годы: станет ли антимикробная фотодинамическая терапия (АФДТ) альтернативой стандартному лечению локализованных инфекций в той мере, в какой этот метод был принят медицинским сообществом при лечении поверхностных опухолей. Наши и многие другие исследования, описанные в данном обзоре, действительно показывают, что АФДТ имеет хороший потенциал, чтобы занять важное место среди ряда методов уничтожения резистентных микробов. Однако многое, если не все, будет зависеть от появления когорты врачей, готовых принять эту новую парадигму. В обзоре рассмотрены природные хлориновые фотосенсибилизаторы (ФС), используемые в АФДТ. Представлены соответствующие молекулярные структуры макрогетероциклов, сопоставлен и кратко обсужден ряд важных результатов по фотодинамической инактивации как музейных, так и нозокомиальных антибиотикорезистентных микроорганизмов in vitro. Проанализированы возможные пути потенцирования антимикробной активности хлориновых ФС путем использования соединений не макрогетероциклической природы. Доказано, что катионные макрогетероциклы являются наиболее эффективными агентами для элиминации как грамположительных, так и грамотрицательных патогенов при соответствующем облучении. Среди них важное место занимают производные хлорофилла - они малотоксичны для клеток млекопитающих, разрушаются при облучении и быстро выводятся из организма. Показано, что сильное потенцирование АФДТ путем добавления неорганических солей или полимеров в растворы ФС без роста темновой токсичности будет представлять особый интерес в ближайшие годы. Таким образом, разработка специальных лекарственных форм, содержащих как ФС, так и потенцирующий агент, а также сочетание АФДТ с традиционными методами лечения локализованных инфекций может вывести лечение микробных инфекций на качественно новый уровень.

Ключевые слова: антибиотикорезистентные микроорганизмы, фотодинамическая инактивация, хлорофилловые фотосенсибилизаторы, грамположительные и грамотрицательные бактерии, потенцирующие агенты

NATURAL CHLORIN PHOTOSENSITIZERS AND POTENTIATING AGENTS FOR ANTIMICROBIAL PHOTODYNAMIC THERAPY

A.V. Kustov

Andrey V. Kustov (ORCID 0000-0002-3553-6206)

G.A. Krestov Institute of Solution Chemistry of the RAS, Akademicheskaya st., 1, Ivanovo, 153045, Russia
E-mail: kustov@isuct.ru

Because of the antibiotic era is considered to be on the verge of ending and the probability of discovering novel classes of antibiotics is estimated to be low, it is necessary to discover alternative technologies to fight with antibiotic resistant microoorganisms. The key question to be answered in recent years is: will become APDT the alternative to the standard treatment of localized infections to the extent this modality has been adopted by the medical community in treating superficial tumors. Our and many other studies mentioned here do indicate that APDT has a good potential to take an important place in the arsenal of killing resistant microbes. However, much, if not all, will depend on the appearance of the cohort of physicians willing to accept this new paradigm. In this short review, we focus on the most popular chlorin photosensitizers (PSs) used in antimicrobial photodynamic therapy (APDT). The appropriate molecular structures and several important results of photodynamic inactivation of both archival and nosocomial antibiotic resistant microorganisms are given in some detail and briefly discussed. The possible ways of potentiating antimicrobial activity of chlorophyll-based PSs are also analyzed. It has been proven that cationic macroheterocycles are proved to be the most efficient agents for eliminating both Gram-positive and Gram-negative pathogens under appropriate irradiation. Among them chlorophyll derivatives are found to be low toxic to mammalian cells, are destroyed under irradiation and rapidly removed from the body. It has been shown that strong potentiation of APDT by adding inorganic salts or polymers to PS solutions without the growth of dark toxicity will be of special interest in the next years. The development of special dosage forms containing both a PS and a potentiating agent as well as the combination of APDT with traditional methods of treating localized infections may provide additional benefit for many patients.

Key words: antibiotic resistant microorganisms, photodynamic inactivation, chlorophyll photosensitizers, Gram-positive and Gram-negative bacteria, potentiating agents

Для цитирования:
Кустов А.В. Натуральные хлориновые фотосенсибилизаторы и потенцирующие агенты для антимикробной фотодинамической терапии. *Изв. вузов. Химия и хим. технология*. 2023. Т. 66. Вып. 12. С. 32–40. DOI: 10.6060/ivkkt.20236612.6902.

For citation:
Kustov A.V. Natural chlorin photosensitizers and potentiating agents for antimicrobial photodynamic therapy. *ChemChemTech [Izv. Vyssh. Uchebn. Zaved. Khim. Khim. Tekhnol.]*. 2023. V. 66. N 12. P. 32–40. DOI: 10.6060/ivkkt.20236612.6902.

INTRODUCTION

It is well known that antibiotics are steadily losing their impact to kill pathogenic microorganisms and multi-resistant superbugs are detected both among Gram-positive and Gram-negative bacteria [1-3]. The previously dominated trend [3] that most, if not all, microbial infections may be efficiently treated with antibiotics is gradually becoming a thing of the past, and humanity has finally recognized that pathogenic microorganisms have a unique ability to adapt to adverse environment conditions due to various mutations and biofilm formation [4, 5]. Additionally, inappropriate prescription of antibiotics for viral infections, a low dose or insufficient duration of treating bacterial infections, the pollution of the environment by preservative or antibiotic sewage waters provide further grist to the mill [5].

Several years ago, six superbugs were included into the so-called "ESKAPE" group containing the bacteria with resistance to multiple antibiotic classes [1, 7]. Besides traditional ways proposed to treat resistant localized infections [7, 8], the important role is given to antimicrobial photodynamic therapy [5, 6, 8, 9]. Originally developed to treat superficial tumors (see [9-12] and references therein), APDT is well-established and easily repeatable approach to eliminate various classes of bacteria, fungi, viruses *etc*. [5, 6-8, 13, 14]. It involves a two-step procedure consisting of administrating a light-activated drug (photosensitizer) which is rapidly accumulated in microbial cells followed by local irradiation of an infected area by visible light. This initiates a cascade of photochemical reactions leading to the formation of highly reactive oxygen species (ROS). These ROS execute fatal damage towards microbial cells inducing their efficient killing. It is apparent that this approach may play a crucial role when living in a world without efficient antibiotics in the future.

For the last fifteen years it has been recognized that APDT is an efficient technique to kill both antibiotic-sensitive and multi-resistant bacteria and fungi

[13-19]. The mechanism of a bacteria killing in this case is a multi-target damaging process compared to the antibiotic killing that acts very specifically to a definite target [5, 8, 9]. It is important that for APDT no specific ligand–receptor interaction is necessary as well as no specific extracellular or intracellular localization of a given photosensitizer (PS) is needed [5].

Many types of photosensitizing agents have been synthesized and studied in detail over the past years (see, for example, [6, 9, 13, 18, 20, 21] and references therein). The most popular PSs have a macrocyclic structure and belong to a porphyrin-, phthalocyanine-, chlorin- or bacteriochlorin-type of photosensitizing agents. During the past decade, we have been currently involved in an intensive and continuing series of investigations [12, 15-17, 22-24] dealing with the antimicrobial and antitumor activity of natural chlorophyll PSs. Our interest to these green pigments came from their unique molecular structure and properties. Firstly, these PSs may be easily obtained from natural chlorophyll [15-17]. Secondly, they are safe in the dark and as natural compounds are rapidly removed from the human body [25-28]. Thirdly and generally, these pigments belonging to the second generation of PSs generate singlet oxygen with a good quantum yield [12, 29] and are proved to be efficient agents in treating both malignant tumors [12, 24-27] and localized infections [8, 9, 17, 24].

Here, we focus on some important chlorin-type PSs used in APDT and compare their ability to kill bacteria and fungi *in vitro* both in the dark and under irradiation.

DISCUSSION

We have noted above that many agents in APDT have a macrocyclic structure which is very similar to that of protoporphyrin-IX in human hemoglobin [9, 25]. Chlorins are known to be reduced porphyrins and are obtained by extraction from natural raw materials followed by an appropriate chemical modification or by hydrogenating of one exo-pyrrol double bond in a porphyrin macrocycle. This leads to the shift of the absorption Q-band to larger wavelengths and increases the intensity of red light absorbance by a PS molecule [25, 30-32].

The most popular domestic chlorin PSs in antitumor PDT such as “Fotoditazine”, “Fotoran e_6” or “Radachlorin” contain chlorin e_6 as the main component (see Fig. 1) and eradicate pathogenic microorganisms under irradiation [9, 24, 33, 34]. However, their effect is mainly restricted by Gram-positive bacteria. It is not surprising because more 30 years ago it was found that there was a fundamental difference in susceptibility to APDT between Gram-positive and Gram-negative bacteria [35]. In general, anionic and neutral PSs are efficiently bound to Gram-positive bacterial and fungal cells and photoinactivate them. In contrast, Gram-negative bacteria are relatively resistant to these agents due to low affinity of PS molecules to their outer lipopolysaccharide membrane.

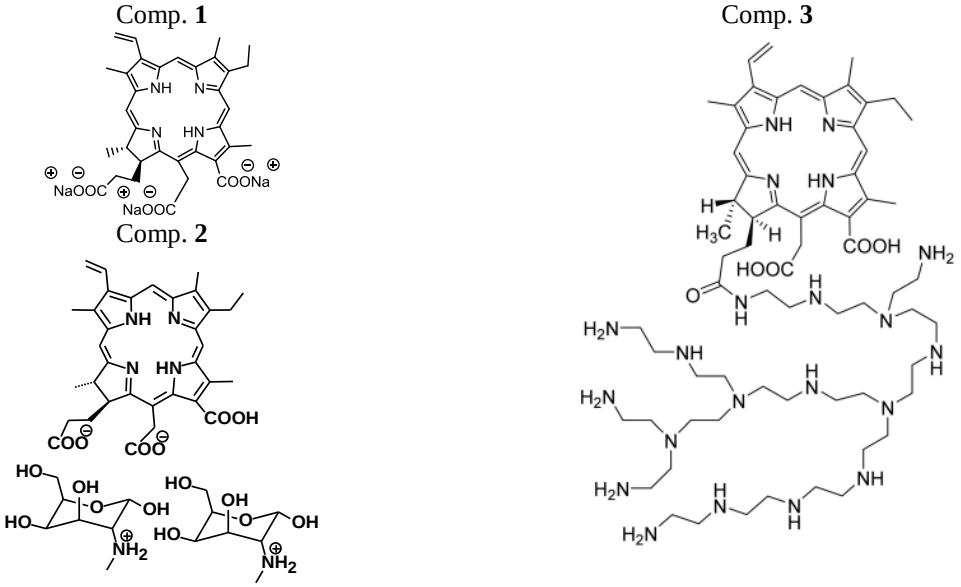

Fig. 1. Molecular structures of the clinically approved chlorin PSs: chlorin e_6 trisodium salt (comp. 1, its 1:1 mixture with polyvinylpyrrolidone is known as "Fotoran e_6"); "Fotoditazin" as a dimeglumine salt of chlorin e_6 (comp. 2) and the conjugate of chlorin e_6 with polyethylenimine (comp. 3) used for treating endodontic infections [13]

Рис. 1. Молекулярные структуры клинически одобренных хлориновых ФС: тринатриевой соли хлорина e_6 (соед. 1, ее смесь 1:1 с поливинилпирролидоном известна как «Фоторан e_6»); «Фотодитазина» в виде димеглюминовой соли хлорина e_6 (соед. 2) и конъюгата хлорина e_6 с полиэтиленимином (соед. 3), применяемого для лечения эндодонтических инфекций [13]

Fig. 2 compares the dose-dependent killing curves for chlorin e_6 and its cationic conjugate with polyethyleneimine (see Fig. 1) at the PS concentration of 10 μM [13, 36]. We see that the polymer construct is highly efficient in mediating APDT of Gram-positive and Gram-negative bacteria. The complete elimination of *Staphylococcus aureus* is observed at a light dose of 15 J/cm^2, while *Pseudomonas aeruginosa* requires a much higher fluence, *viz*. 40 J/cm^2. It is obvious that the positive charges of the conjugate help it to bind to the negatively charged bacteria wall and its polycationic nature enables the PS to penetrate quickly the outer membrane of Gram-negative cells by disrupting its structure [13]. The important feature of such cationic PSs is their macromolecular nature providing a temporal selectivity to bacterial cells, while mammalian cells take them up by the time-dependent process of endocytosis.

In sharp contrast, anionic chlorin e_6 is found to be much less efficient and gives two-three logs of bacterial killing. Fig. 2 shows that susceptibility to APDT with chlorin e_6 is larger for *Staphylococcus aureus*. However, the effect is rather weak at this low PS concentration. The increase in a chlorin e_6 concentration and a light dose provides the complete elimination of *Staphylococcus aureus* [33], while the killing of nosocomial strains of *Pseudomonas aeruginosa* does not exceed two-four logs.

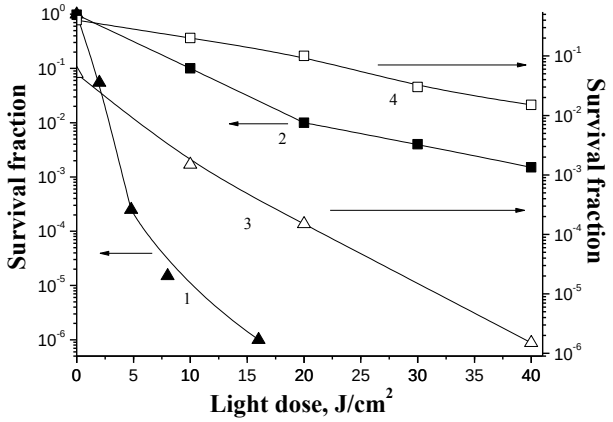

Fig. 2. Photoinactivation of *Staphylococcus aureus* (1, 2; left-hand scale) and *Pseudomonas aeruginosa* (3, 4; right-hand scale) by comp. **1** (2, 4) and comp. **3** (1, 3) [13, 36]. The PS concentration and incubation time were 10 μM and 10 min, respectively. Lines are spline functions

Рис. 2. Фотоинактивация *Staphylococcus aureus* (1, 2; левая шкала) и *Pseudomonas aeruginosa* (3, 4; правая шкала) соед. **1** (2, 4) и соед. **3** (1, 3) [13, 36]. Концентрация ФС и время инкубации составляли 10 мкМ и 10 мин, соответственно. Линии – сплайн-функции

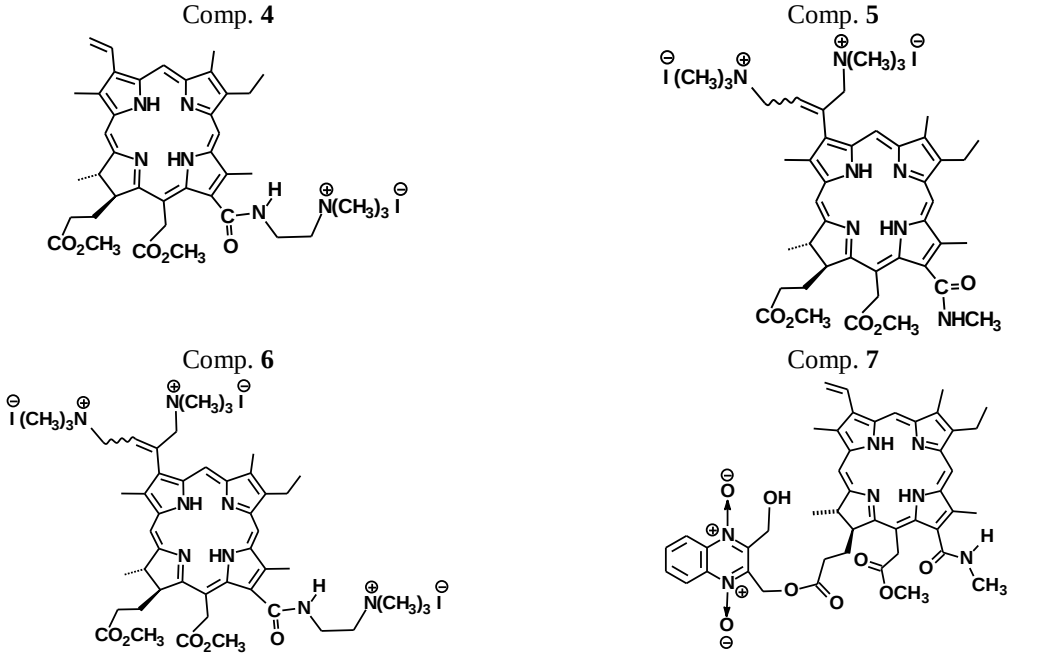

Fig. 3. Molecular structures of mono- (comp. **4**), di- (comp. **5**) and tricationic (comp. **6**) chlorin photosensitizers as well as the conjugate of chlorin e_6 with dioxidine (comp. **7**). The chemical names of PSs can be found in original papers [15, 16, 37]

Рис. 3. Молекулярные структуры моно- (соед. **4**), ди- (соед. **5**) и трикатионных (соед. **6**) хлориновых фотосенсибилизаторов, а также конъюгата хлорина e_6 с диоксидином (соед. **7**). Химические названия ФС даны в оригинальных работах [15, 16, 37]

Recently [15-17, 24] we have studied in some detail photoinactivation of both archival and nosocomial antibiotic resistant pathogens by neutral and several cationic chlorin PSs containing one, two or three trimethylammonium groups (see comps. **4**-**6** in Fig. 3).

The results of the first series of experiments with archival strains was twofold: both mono- and polycationic chlorins provided the efficient killing of *Staphylococcus aureus* and *Candida albicans*, but were ineffective towards *Escherichia coli* (see the first section in Table 1). In sharp contrast, the neutral conjugate of chlorin e_6 with antimicrobial drug "Dioxidine" (comp. 7) showed promising results in mediating APDT of plankton forms of *Escherichia coli* [15]. However, further studies with much larger colony forming unit (CFU) values were not too successful both *in vitro* and *in vivo* [17].

Table 1

Inactivation of archival pathogens by comps. 4-6 in the dark and under irradiation

Таблица 1. **Инактивация архивных патогенов соед. 4-6 в темноте и при облучении**

Pathogen	Survived microbial cell numbers					
	Dark			Irradiation, 40 J/cm^2		
	Comp. 4	Comp. 5	Comp. 6	Comp. 4	Comp. 5	Comp. 6
	1. Initial bacterial cell number was 10^3, m_{PS} = 0.00005 mol/kg [15]					
Staphylococcus aureus	980	30	0	0	0	0
Candida albicans	0	0	40	0	0	0
Escherichia coli	1000	1000	1000	1000	1000	1000
	2. Initial bacterial cell number was 10^7, m_{PS} = 0.0001 mol/kg + 0.1 mass. % Na_2H_2Edta [16]					
Staphylococcus aureus	0	0	0	0	0	0
Escherichia coli	0	0	0	0	0	0
Candida albicans	0	0	0	0	0	0
	3. Initial bacterial cell number was 10^7, m_{PS} = 0.0005 mol/kg + 1 mass. % Tween 80 [16]					
Staphylococcus aureus	0 [a]	0	-	0 [a]	0	-
Escherichia coli	$1.5\ 10^6$ [a]	10^7	0	0 [a]	0	0
Candida albicans	0 [a]	$7.5\ 10^6$	-	0 [a]	$5\ 10^5$	-

Note: [a] – solutions contained 0.1 mass. % of Na_2H_2Edta

Примечание: [a] – растворы содержали 0,1 мас. % Na_2H_2Edta

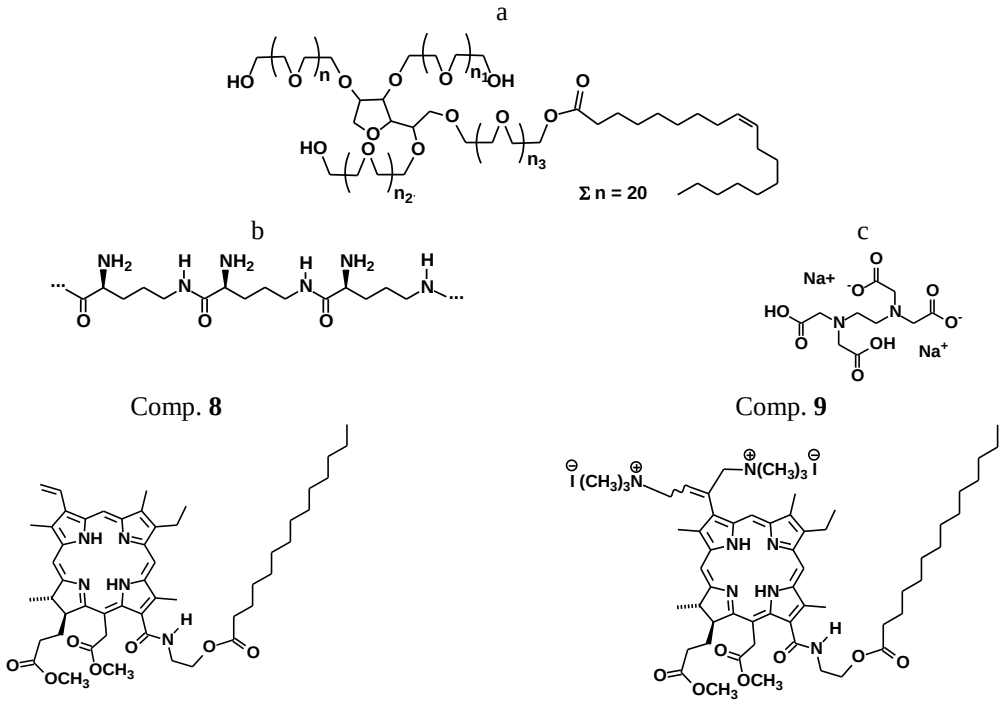

Fig. 4. Molecular structures of potentiating agents and conjugates with myristic acid: (a) Tween 80, (б) ε-polylysine N~30, (c) disodium ethylene diaminetetraacetate (Na_2H_2Edta); neutral (comp. **8**) and dicationic (comp. **9**) conjugates of chlorin e_6 with myristic acid. The chemical names of PSs are given elsewhere [17]

Рис. 4. Молекулярные структуры потенциирующих агентов и конъюгатов с миристиновой кислотой: (a) Tween 80, (б) ε-полилизин N~30, (c) динатрийэтилендиаминтетраацетат (Na_2H_2Edta); нейтральные (соед. **8**) и дикатионные (соед. **9**) конъюгаты хлорина e_6 с миристиновой кислотой. Химические названия ФС приведены в [17]

As for the cationic PSs mentioned above, we may state that either the intrinsic charge of cationic PSs was insufficient to penetrate the outer membrane of *Escherichia coli* or a PS concentration and a light dose were too small to inactivate Gram-negative bacteria. The similar findings were made for chlorin e_6 aminobutyl amide and its monocationic derivative [38]. Thus, in the further experiments with cationic chlorins we used higher PS concentrations and added to a PS solution appropriate potentiating agents such as Na_2H_2Edta or Twin 80 (see Fig. 4). This approach is seen to improve the results and leads to complete elimination of all the three pathogens.

The mechanism of Na_2H_2Edta toxicity is well known and consists of destabilization of the outer membrane *via* calcium chelation. Twin 80 utilizes another way. This safe non-ionic surfactant [39] forms spherical micelles [40, 41] efficiently solubilizing hydrophobic chlorin molecules. This prevents PS hydrophobic association and strongly increases generation of singlet oxygen, which seems to be responsible for enhancing toxicity under irradiation. It is worthy of note that some potentiating agents have their own toxicity towards bacterial cells or are able to enhance toxicity of PS molecules. Table 2 compares dark toxicity of aqueous solutions of several potentiating agents to archival and nosocomial antibiotic resistant microorganisms. We see that diluted solutions of Na_2H_2Edta reveal dark toxicity towards *Staphylococcus aureus* and *Candida albicans*, while Gram-negative bacteria, *viz*. *Escherichia coli* and *Pseudomonas aeruginosa*, are resistant to Na_2H_2Edta up to 0.5 mass % of the electrolyte. However, addition of 0.1 mass % of Na_2H_2Edta to diluted PS solutions (see Section 2 in Table 1) leads to complete elimination of *Escherichia coli* in the dark and there seems to be some synergetic effect enhancing toxicity of Na_2H_2Edta and PS molecules to bacterial cells. Fig. 1 shows that the behavior of the cationic conjugate with polyethyleneimine is similar and is accompanied by much larger dark toxicity towards *Pseudomonas aeruginosa* compared to pure chlorin e_6.

Table 2

Inactivation of archival and nosocomial pathogens by potentiating agents in the dark

***Таблица 2.* Инактивация архивных и внутрибольничных возбудителей потенцирующими агентами в темноте**

Microorganism	Survived microbial cell numbers[a]		
Archival strains [16]	Tween 80, 1%	Na_2H_2Edta, 0.5%	Na_2H_2Edta, 0.2%
Staphylococcus aureus	10^7	0	0
Escherichia coli	10^7	0	10^7
Candida albicans	10^7	0	0
Nosocomial antibiotic resistant strains	Pl, 0.1%	Pl, 0.05%	Na_2H_2Edta, 0.05%
Pseudomonas aeruginosa	10^7	10^7	10^7

Note: [a] – the initial CFU number was 10^7, the incubation time was 24 h

Примечание: [a] – исходное количество КОЕ 10^7, время инкубации 24 ч

The conjugation of chlorin e_6 with myristic acid (see comps. **8**, **9** in Fig. 4) as a key fragment of the well-known antibiotic drug "Miramistin" [42] was directed towards overcoming the resistance of Gram-negative pathogens to APDT [17]. Archival strains were found to be highly sensitive to a PS concentration of 0.5 mM both in the dark and under irradiation. The fivefold decrease in a pigment content gives true photodynamic inactivation with the dose of 40 J/cm^2 [17]. Antibiotic resistant nosocomial strains and, especially, *Escherichia coli*, were more resistant to photoinactivation with comps. **8**, **9** and required twice as much the light fluence for complete elimination. This unique resistance of *Escherichia coli* to APDT has been noted several times before [15, 43] and should be the subject of the future studies.

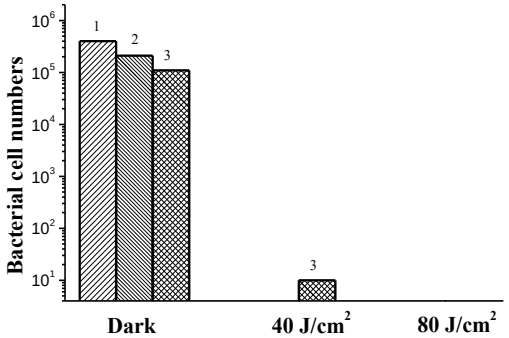

Fig. 5. Photoinactivation of Gram-negative bacteria *Pseudomonas aeruginosa* (1), *Enterobacter cloacae* (2), *Acinetobacter baumannii* (3) by monocationic chlorin (comp. **4**). The PS molality was 100 (1), 25 (2) and 50 (3) μmol/kg, respectively [34]

Рис. 5. Фотоинактивация грамотрицательных бактерий *Pseudomonas aeruginosa* (1), *Enterobacter cloacae* (2), *Acinetobacter baumannii* (3) монокатионным хлорином (соед. **4**). Моляльность ФС составляла 100 (1), 25 (2) и 50 (3) мкмоль/кг, соответственно [34]

Although both Gram-positive and Gram-negative are highly susceptible to photoinactivation with the conjugates mentioned above, comps. **8**, **9** are almost insoluble in water, which requires the use of appropriate carriers [17]. Additionally, synthesis and purification of these pigments are of costly enough. Monocationic chlorin (comp. **4**) mentioned above shows a comparable photodynamic activity [34] and seems to be an appropriate PS in APDT. Fig. 5 compares photodynamic inactivation of several Gram-negative bacteria belonging to the ESKAPE group. We see that incubation in the dark for 40 minutes does not lead to any significant decrease in the CFU value, while irradiation with the dose of 40 J/cm^2 gives four-six logs of killing. The light fluence of 80 J/cm^2 gives complete elimination.

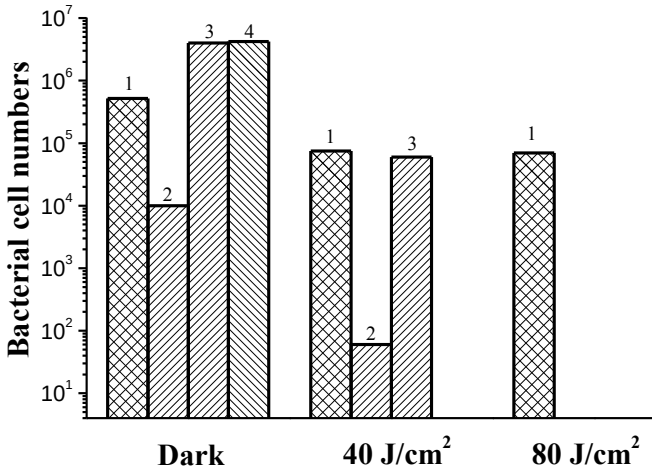

Fig. 6. Photoinactivation of Gram-negative bacteria *Enterobacter cloacae* (1, 2) and *Pseudomonas aeruginosa* (3, 4) by chlorin e_6 trisodium salt (comp. **1**). 1 – 50 μmol/kg, 2 – 100 μmol/kg+0.05% of Pl, 3 – 100 μmol/kg + 0.1% of Pl, 4 – 100 μmol/kg + 0.05% of Na_2H_2Edta

Рис. 6. Фотоинактивация грамотрицательных бактерий *Enterobacter cloacae* (1, 2) и *Pseudomonas aeruginosa* (3, 4) тринатриевой солью хлорина e_6 (соед. **1**). 1 – 50 мкмоль/кг, 2 – 100 мкмоль/кг + 0,05% Pl, 3 – 100 мкмоль/кг + 0,1% Pl, 4 – 100 мкмоль/кг + 0,05% Na_2H_2Edta

Another approach comes from intriguing possibility of PS molecules to enhance bacterial killing in an aqueous solution of potentiating agents such as several inorganic salts, polymers or proteins [9, 14]. We added 1-5 mM of KI to a 0.1 mM solution of chlorin e_6 but any potentiation of killing was not observed. It is apparent that either the iodide-ion concentration used was insufficient to give an appropriate amount of reactive iodide species/molecular iodine to kill bacteria or KI is not appropriate potentiating agent for anionic chlorin PSs. In contrast, Fig. 6 clearly shows that photodynamic inactivation is strongly enhanced by addition of a small amount of Na_2H_2Edta or ε-polylysine.

CONCLUSIONS

Because of the antibiotic era is considered to be on the verge of ending and the probability of discovering novel classes of antibiotics is estimated to be low [13], it is necessary to discover alternative technologies to fight with antibiotic resistant microorganisms. The key question to be answered in recent years is: will become APDT the alternative to the standard treatment of localized infections to the extent this modality has been adopted by the medical community in treating superficial tumors. Our and many other studies mentioned here do indicate that APDT has a good potential to take an important place in the arsenal of killing resistant microbes. However, much, if not all, will depend on the appearance of the cohort of physicians willing to accept this new paradigm.

In conclusion of this short review, we would like to make several important findings, as a result of the present and several earlier investigations using macrocyclic photosensitizers [13-17, 23, 41, 43] as potential agents for PDT. (a) Cationic macroheterocycles are proved to be the most efficient agents for eliminating both Gram-positive and Gram-negative pathogens under appropriate irradiation. Among them chlorophyll derivatives are found to be low toxic to mammalian cells, are destroyed under irradiation and rapidly removed from the body. (b) The strong potentiation of APDT by adding inorganic salts or polymers to PS solutions without the growth of dark toxicity will be of special interest in the next years. The development of special dosage forms containing both a PS and a potentiating agent as well as the combination of APDT with traditional methods of treating localized infections may provide additional benefit for many patients. (c) This seems to be clear, if not precise, that the antimicrobial efficacy of usual antitumor PSs, *i.e.* "Fotoran e_6" or "Fotoditazin" may be enhanced by adding a small amount of low toxic Na_2H_2Edta or ε-polylysine. The former chelates calcium and destroys the outer membrane, while the latter seems to form ionic complexes with negatively charged chlorins. This entity efficiently binds to a bacterial wall giving ROS execute fatal damage towards microbial cells. It will, however, necessary to study this effect in some detail to develop the optimum procedure of the application of this approach both *in vitro* and *in vivo*.

ACKNOWLEDGEMENTS

The author is grateful to post-graduate student N.V. Kukushkina (ISC of RAS) and physician N.N. Solomonova (Ivanovo State Regional Hospital) for providing a series of microbiological studies.

This research was funded by RFBR and Ivanovo Region, project 20-415-37002.

The authors declare the absence a conflict of interest in this article.

Автор благодарен аспирантке Н.В. Кукушкиной (ИНЦ РАН) и врачу Н.Н. Соломоновой (Ивановская областная клиническая больница) за проведение микробиологических исследований.

Исследование выполнено при финансовой поддержке РФФИ и Ивановской области, проект 20-415-37002.

Авторы заявляют об отсутствии конфликта интересов в данной статье.

REFERENCES
ЛИТЕРАТУРА

1. WHO. WHO Library Cataloguing-in-Publication Data. Antimicrobial resistance: global report on surveillance. 2014. P. 1–232.
2. **Laxminarayan R., Matsoso P., Pant S., Brower C., Røttingen J-A., Klugman K., Davies S.** // *Lancet.* 2016. V. 387. P. 168–175. DOI: 10.1016/S0140-6736(15)00474-2.
3. **Banin E., Hughes D., Kuipers O.P.** // *FEMS Microbiol. Rev.* 2017. V. 41. P. 450-452. DOI: 10.1093/femsre/fux016.
4. **Biel M.A.** Photodynamic therapy of bacterial and fungal biofilm infections. In: Photodynamic Therapy. Methods and Protocols. Ed. by C.J. Gomer. Berlin: Springer Dordrecht Heidelberg London. 2010. P. 175-94. DOI: 10.1007/978-1-60761-697-9_13.
5. **Maisch T.** // *Photochem. Photobiol. Sci.* 2015. V. 14. P. 1518-1526. DOI: 10.1039/c5pp00037h.
6. **Yao L., Rong Q., Zaat S.A.J., Breukink E., Heger M.** // *J. Clin. Transl. Res.* 2015. V. 1. P. 140-67. DOI: 10.18053/jctres.201503.002.
7. **Czaplewski L., Bax R., Clokie M., Dawson M., Fairhead H.** // *Lancet Infect. Dis.* 2016. V. 16. P. 239-51. DOI: 10.1016/S1473-3099(15)00466-1.
8. **Wainwright M., Maisch T., Nonell S., Plaetzer K., Almeida A., Tegos G.P., Hamblin M.R.** // *Lancet Infect. Dis.* 2017. V. 17. P. e49-e55. DOI: 10.1016/S1473-3099(16)30268-7.
9. **Kustov A.V., Berezin D.B., Strelnikov A.I., Lapochkina N.P.** Antitumor and Antimicrobial Photodynamic Therapy: Mechanisms, Targets, Clinical and Laboratory Studies: a Guide. Ed. by A.K. Gagua. M.: Largo. 2020. 108 p. (in Russian).
Кустов А.В., Березин Д.Б., Стрельников А.И., Лапочкина Н.П. Противоопухолевая и антимикробная фотодинамическая терапия: механизмы, мишени, клинико-лабораторные исследования. Практическое руководство. Под ред. А.К. Гагуа. М.: Ларго. 2020. 108 с.
10. **Bonnett R.** Chemical aspects of photodynamic therapy. London: VHC Publ. 2000. DOI: 10.1201/9781482296952.
11. **Van Straten D., Mashayekhi V., de Bruijn H.S., Oliveira S., Robinson D.J.** // *Cancers.* 2017. V. 9. P. 1–54. DOI: 10.3390/cancers9020019.
12. **Kustov A.V., Privalov O.A., Strelnikov A.I., Koifman O.I., Lubimtsev A.V.** // *J. Clin. Med.* 2022. V. 11. P. 233. DOI: 10.3390/jcm11010233.
13. **Hamblin M.R.** // *Curr. Opin. Microbiol.* 2016. V. 33. P. 67–73. DOI: 10.1016/j.mib.2016.06.008.
14. **Hamblin M.R.** // *Expert. Rev. Anti. Infect. Ther.* 2017. V. 15. P. 1059–1069. DOI: 10.1080/14787210.2017.1397512.
15. **Kustov A.V., Garas'ko E.V., Belykh D.V., Khudyaeva I.S., Startseva O.M., Makarov V.V., Strel'nikov A.I., Berezin D.B.** // *Uspekhi Sovrem. Estestvozn.* 2016. V. 12. P. 263-268 (in Russian). DOI: 10.17513/use.36297.
Кустов А.В., Гарасько Е.В., Белых Д.В., Худяева И.С., Старцева О.М., Макаров В.В, Стрельников А.И., Березин Д.Б. // *Усп. соврем. естествозн.* 2016. N 12. P. 263-268.
16. **Kustov A.V., Belykh D.V., Smirnova N.L., Venediktov E.A., Kudayarova T.V.** // *Dye. Pigment.* 2018. V. 149. P. 553–559. DOI: 10.1016/j.dyepig.2017.09.073.
17. **Kustov A.V., Kustova T.V., Belykh D.V., Khudyaeva I.S., Berezin D.B.** // *Dye. Pigment.* 2020 V. 173. P. 107948. DOI: 10.1016/j.dyepig.2019.107948.
18. **Suvorov N., Pogorilyy V., Diachkova E., Vasil'ev Y., Mironov A., Grin M.** // *Int. J. Mol. Sci.* 2021. V. 22. P. 6392. DOI: 10.3390/ijms22126392.
19. **Berezin D.B., Kruchin S.O., Kukushkina N.V., Venediktov E.A., Koifman M.O., Kustov A.V.** // *Photochem.* 2023. V. 3. P. 171–186. DOI: 10.3390/photochem3010011.
20. **Kikuchi T., Mogi M., Okabe I., Okada K., Goto H.** // *Int. J. Mol. Sci.* 2015. V. 16. P. 24111-24126. DOI: 10.3390/ijms 161024111.
21. **Cieplik F., Deng D., Crielaard W., Buchalla W., Hellwig E., Al-Ahmad A., Maisch T.** // *Crit. Rev. Microbiol.* 2018. V. 44. P. 571-589. DOI: 10.1080/1040841X.2018.1467876.
22. **Caterino M., D'Aria F., Kustov A.V., Belykh D.V., Khudyaeva I.S.** // *Int. J. Biol. Macromol.* 2020. V. 145. P. 244–251. DOI: 10.1016/j.ijbiomac.2019.12.152.
23. **Kustov A.V., Morshnev Ph.K., Kukushkina N.V., Smirnova N.L., Berezin D.B.** // *Int. J. Mol. Sci.* 2022. V. 23. P. 5294. DOI: 10.3390/ijms23105294.
24. **Kustov A.V., Berezin D.B., Zorin V.P., Morshnev P.K., Kukushkina N.V.** // *Pharmaceutics.* 2023. V. 15. P. 61. DOI: 10.3390/pharmaceutics15010061.
25. **Brandis A.S., Salomon Y., Schetz A.** Chlorophyll sensitizers in photodynamic therapy. In: Chlorophylls and bacteriochlorophylls: biochemistry, biophysics, functions and application. Ed. by B. Grimm, R.J. Porra, W. Rüdiger, H. Scheer. Berlin: Springer. 2006. P. 461-83. DOI: 10.1007/1-4020-4516-6_32.
26. **Trukhacheva T.V., Shlaykhtin S.V., Novikov G.A., Istomin Yu.A.** Fotolon (Photolon) – a new photosensitizer for photodynamic therapy. Minsk: RUP Belmedpreparaty. 2006. 64 p. (in Russian).
Трухачева Т.В., Шляхтин С.В., Исаков Г.А., Истомин Ю.П. Фотолон - новое средство для фотодинамической терапии. Обзор результатов фармацевтических, фармакологических и клинических исследований. Минск: РУП Белмедпрепараты. 2009. 62 с.
27. **Lee J.Y., Diaz R.R., Cho K.S., Lim M.S., Chung J.S., Kim W.T., Ham W.S., Choi Y.D.** // *J. Urol.* 2013. V. 190. P. 1192–1199. DOI: 10.1016/j.juro.2013.04.077.
28. **Zhidomorov N.Yu., Nazarenko O.A., Demidov V.I., Kustov A.V., Kukushkina N.V.** // *Biomed. Photonics.* 2022. V. 11. P. 23–32. DOI: 10.24931/2413–9432–2022–11-2-23-32.
Жидоморов Н.Ю., Назаренко О.А., Демидов В.И., Кустов А.В., Кукушкина Н.В., Койфман О.И., Гагуа А.К., Томилова И.К., Березин Д.Б. // *Biomed. Photonics.* 2022. 11. № 2. С. 23–32.

29. **Kustov A.V., Morshnev Ph.K., Kukushkina N.V., Krestyaninov M.A., Smirnova N.L.** // *Comp. Rend. Chim.* 2022. V. 25. P. 97–102. DOI: 10.5802/crchim.158.
30. **Giovannetti R.** The use of spectrophotometry UV-Vis for study of porphyrins. In: Nanotechnology and nanomaterials. Micro to nano spectroscopy. Ed. by J. Uddin. Rijeka: In Tech Europe. 2012. P. 87-108. DOI: 10.5772/38797.
31. **Shukhto O.V., Khudyaeva I.S., Belykh D.V., Berezin D.B.** // *ChemChemTech [Izv. Vyssh. Uchebn. Zaved. Khim. Khim. Tekhnol.]*. 2021. V. 64. N 11. P. 86-96.
Шухто О.В., Березин Д.Б., Худяева И.С., Белых Д.В. // *Изв. вузов. Химия и хим. технология.* 2021. Т. 64. Вып. 11. С. 86-96. DOI: 10.6060/ivkkt.20216411.6500.
32. **Zenkevich E., Sagun E., Knyukshto V., Schulga A., Mironov A.** // *Photochem. Photobiol. B Biology*. 1996. V. 33. P. 171-180. DOI: 10.1016/1011-1344(95)07241-1.
33. **Drulis−Kawa Z., Bednarkiewicz A., Bugla G., Stręk W., Doroszkiewicz W.** // *Adv. Clin. Exp. Med.* 2006. V. 15. P. 279–283.
34. **Kustov A.V., Kukushkina N.V., Lyalyakina E.V., Solomonova N.N., Gagua A.K., Koifman O.I., Berezin D.B.** Monocationic chlorin as a photosensitizer for efficient photoinactivation of Gram-negative antibiotic resistant microorganisms. *Dokl. Phys. Chem.* 2023. V. 508. P. 59-63 (in Russian). DOI: 10.31857/S2686953523700164.
35. **Malik Z., Ladan H., Nitzan Y.** // *J. Photochem. Photobiol. B.* 1992. V. 14. P. 262–266. DOI: 10.1016/1011-1344(92)85104-3.
36. **Tegos G.P., Anbe M., Yang C., Demidova T.N., Satti M.** // *Antimicrob. Agents Chemother*. 2006. V. 50. P. 1402-1410. DOI: 10.1128/AAC.50.4.1402-1410.2006.
37. **Kustov A.V., Belykh D.V., Smirnova N.L., Khudyaeva I.S., Berezin D.B.** // *J. Chem. Thermodyn.* 2017. V. 115. P. 302–306. DOI: 10.1016/j.jct.2017.07.031.
38. **Brusov S.S., Efremenko A.V., Lebedeva V.S., Shchepelina E.Y., Ponomarev Ph.V., Feofanov A.V., Mironov A.F., Grin M.A.** // *Russ. Biother. J.* 2015. V. 14. P. 87–92. DOI: 10.17650/1726-9784-2015-14-4-87-92.
39. **Schwartzberg L.S., Navari R.M.** // *Adv. Ther.* 2018. V. 35. P. 754–767. DOI: 10.1007/s12325-018-0707-z.
40. **Amani A., York P., de Waard H., Anwar J.** // *Soft Matter*. 2011. V. 7. P. 2900-2908. DOI: 10.1039/C0SM00965B.
41. **Kustov A.V., Morshnev Ph.K., Shukhto O.V., Smirnova N.L., Kukushkina N.V., Koifman O.I., Berezin D.B.** // *Izv.. AN Ser. Khim.* 2023. V. 72. P. 566-573 (in Russian).
Кустов А.В., Моршнев Ф.К., Шухто О.В., Смирнова Н.Л., Кукушкина Н.В., Койфман О.И., Березин Д.Б. // Изв. АН. Сер. Хим. 2023. Т. 72. № 2. С. 566-573. DOI: 10.1007/s11172-023-3820-2.
42. **Fromm-Dornieden C., Rembe J.D., Schäfer N., Böhm J., Stuermer E.K.** // *J. Med. Microbiol.* 2015. V. 64. P. 407-414. DOI: 10.1099/jmm.0.000034.
Grin M.A., Brusov S.S., Shchepelina E.Y., Ponomarev P.V., Khrenova M.K., Smirnov A.S., Lebedeva V.S., Mironov A.F. // *Mendeleev Commun.* 2017. V. 4. P. 338-340. DOI: 10.1016/j.mencom.2017.07.005.
43. **Likhonina A.E., Berezin M.B., Krest'yaninov M.A., Berezin D.B.** // *ChemChemTech [Izv. Vyssh. Uchebn. Zaved. Khim. Khim. Tekhnol.]*. 2021. V. 64. N 10. P. 29-39. DOI: 10.6060/ivkkt.20216410.6464.
Лихонина А.Е., Березин М.Б., Крестьянинов М.А., Березин Д.Б. // *Изв. вузов. Химия и хим. технология.* 2021. Т. 64. Вып. 10. С. 29-39. DOI: 10.6060/ivkkt.20216410.6464.

Поступила в редакцию 18.05.2023
Принята к опубликованию 13.06.2023

Received 18.05.2023
Accepted 13.06.2023