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PHOTOINACTIVATION OF GRAM-POSITIVE AND GRAM-NEGATIVE MICROORGANISMS BY CATIONIC PORPHYRINS

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The effectiveness of new cationic porphyrins and their Zn-complexes (6 compounds) was tested against two Gram-positive (*St. aureus* and *St. epidermidis*) and two Gram-negative (*E. coli* K-12 and *Salmonella sp*.) bacteria. It is shown that cationic porphyrins have high photodynamic activity against of both types of microorganisms.

Cationic porphyrins – photodynamic inactivation – Gram-positive and Gramvnegative bacteria

Փորձարկվել է նոր կատիոնային պորֆիրինների և նրանց Zn- համալիրների (6 միացություններ) արդյունավետությունը երկու գրամդրական (*St. aureus* և *St. epidermidis*) և երկու գրամբացասական (*E. coli* K-12 և *Salmonella sp*.) մանրէների նկատմամբ: Ցույց է տրվել, որ երկու տեսակի մանրէների դեմ էլ կատիոնային պորֆիրիններն ունեն բարձր ֆոտոդինամիկական ակտիվություն:

Կատիոնային պորֆիրիններ – ֆոտոդինամիկական ակտիվություն – գրամդրական և գրամբացասական մանրէներ

Эффективность новых катионных порфиринов и их Zn-комплексов (6 соединений) была проверена против двух грамположительных (*St. aureus* and *St. epidermidis*) и двух грамотрицательных (*E. coli* K-12 and *Salmonella sp*.) бактерий. Показано, что катионные порфирины имеют высокую фотодинамическую активность против обоих типов микроорганизмов.

Катионные порфирины – фотодинамическая инактивация – грамположительные и грамотрицательные бактерии

Incremental increase in strains resistant to antibiotics and antiseptics caused great interest in investigations of alternative antibacterial strategies [5, 8]. Currently photodynamic inactivation of microorganisms (PDI) is the most promising direction for the destruction of various bacteria [5, 14]. The concept of photodynamic inactivation of microorganisms follows the principles of photodynamic therapy (PDT) of tumors [5, 6]: non-toxic dyes – photosensitizers can localizes in/on cells, activates by light, to generate singlet oxygen and free radicals, that are toxic to the target cells (microorganisms). PDI has been successfully applied against gram-positive organisms, but most of photosensitizers weakly effect on gram-negative bacteria [7, 9, 10]. Among a large class of photosensitizers a cationic (porphyrins) are the most effective molecules used in photodynamic therapy (PDT) of tumors and photodynamic inactivation (FCI) of microorganisms [2, 6, 7, 14].

In the present study we investigated efficiency of 6 different cationic porphyrins and metalloporphyrins with various peripheral groups against of Gram-positive (*Staphylococcus aureus, Staphylococcus epidermidi*s) and Gram-negative (E.coli, Salmonella sp.) bacteria interesting from a clinical point of view. The purpose of this study was to evaluate the most effective cationic porphyrins with high photosensitizing activity against Gram-positive and Gram-negative microorganisms.

Materials and methods. *Microorganisms.* Gram-positive bacteria (*Staphylococcus aureus, Staphylococcus epidermidis*) were kindly supplied by the A. Alexanyan Institute of Epidemiology, Virology and Medical Parasitology (Yerevan, Armenia). Gram-negative bactera (*Escherichia coli* K-12 strain and *Salmonella sp*.) were used from the collection of microorganisms of Science and Production Center "Armbiotechnology" of NAS of Armenia. Microorganisms were grown in accordance with the procedure of the work [4].

The optical density (OD) of cell suspension was measured with a spectrophotometer Shimadzu UV-VISIBLE Recording Spectrophotometer UV-2100 (Japan) at a wavelength λ = 420 and 670 nm.

Photosensitizers. In this work we used the following porphyrins and metalloporphyrins: meso-tetra-[4-N-(2`-oxyethyl) pyridyl] porphyrin (TOE4PyP), Zn-TOE4PyP; meso-tetra-[4-N-(2`-butyl) pyridyl] porphyrin (TBut4PyP), Zn-TBut4PyP; meso-tetra-[4-N-(2`-allyl) pyridyl] porphyrin (TAll4PyP), Zn-TAll4PyP, which were synthesized in the Yerevan State Medical University [1, 13] and kindly provided for use in the study.

Cytotoxicity and phototoxicity of porphyrins. Porphyrins and metalloporphyrins cytotoxicity was assessed by determining of their minimum cytotoxic and minimum inhibitory concentration for all four microorganisms. Cytotoxicity and phototoxicity of porphyrins was determined according to the methods of work [4].

Statistical analysis. The statistical parameters (average values, standard deviation) used in the experiments were calculated using the program Excel.

Results and Discussion. *Cytotoxicity of photosensitizers.* At the first stage of experiments were obtained minimal cytotoxicity and minimal inhibitory concentrations (MIC) of new cationic porphyrins and their Zn-complexes against Gram-negative bacteria E.coli K-12 in the absence of light. This microorganism is a convenient model for studying the efficiency of cationic porphyrins causing considerable resistance of bacteria toward many neutral and anionic photosensitizers [11]. Among the investigated compounds Zn-TAll4PyP and Zn-TBut4PyP metalloporphyrins have the highest cytotoxicity. The minimum cytotoxic concentration was equal to 1 μg/ml for Zn-metalloporphyrins and 10 μg/ml for metal-free porphyrins. The minimum inhibitory concentration for Zn-TAll4PyP and Zn-TBut4PyP was about 200 μg/ml, for the remaining four compounds it was much higher. High efficiency of allyl and butyl substituted porphyrin derivatives may be attributed, possibly, to their higher lipophilicity, ability to easily penetrate through membrane in comparison to porphyrins with more hydrophilic oxyethyl group (with OH group). High efficiency of their Zn-complexes also probably is determined by metal–phosphate coordination [12].

Phototoxicity of cationic porphyrins. The phototoxic action against of microorganism *E. coli* has been investigated for six derivatives of porphyrin at minimal cytotoxic concentration. Percent of cell survival of *E. coli* K-12 depending of the concentration of various porphyrins represented in Figure 1.

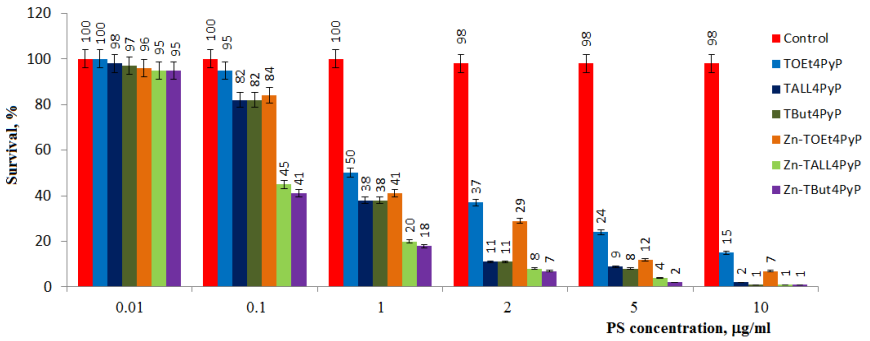

Figure 1. Phototoxic action of various cationic porphyrins against the microorganism *E. coli* K-12.

Figure 1 shows that all of the Zn-metalloporphyrins are more phototoxic in comparison with the same metal-free porphyrins. Zn-TBut4PyP and Zn-TAll4PyP metalloporphyrins possess the highest photodynamic activity among the investigated compounds. This comparison also correlated with the results of our co-workers (Institute of Physics NAS of Belarus, Minsk), from which it follows that the same Zn-metalloporphyrins have a significantly higher quantum yield of singlet oxygen (γΔ) compared to metal-free porphyrins (γΔ= 75 % for TOE4PyP, γΔ= 77 % for TAll4PyP and γΔ= 79 % for TBut4PyP, γΔ= 85 % for Zn-TOE4PyP, γΔ= 86 % for Zn-TAll4PyP and γΔ= 97 % for Zn-TBut4PyP) [3]. Thus, it follows that not only the structure of porphyrins, but also the generation of singlet oxygen are essential to the effectiveness of the photosensitizer.

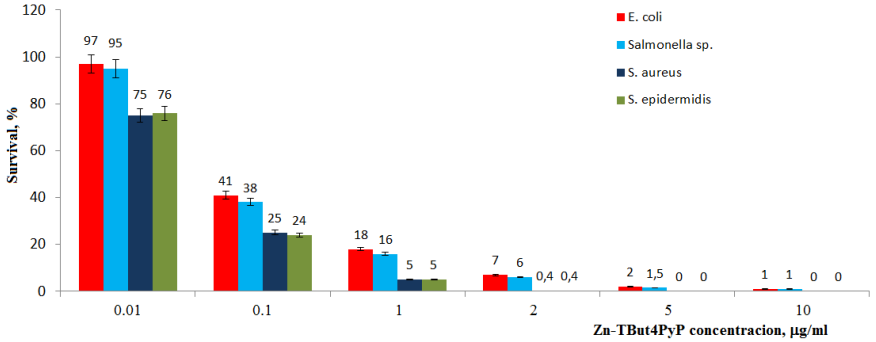

Figure 2. Photodynamic action of Zn-TBut4PyP against Gram-positive (*St. aureus* and *St. epidermidis*) and Gram-negative (*E.coli*, strain K -12, and *Salmonella sp*.) microorganisms.

In further experiments Zn-TBut4PyP photosensitizer was selected to determine photodynamic inactivation of Gram-positive and Gram-negative microorganisms. Experiments results are given in Figure 2. Gram-positive microorganisms are 3-5 times more susceptible to the PDI by Zn-TBut4PyP than Gram-negative microorganisms as expected. Thus, Zn-containing new cationic metalloporphyrins have a high photodynamic activity against both Gram-positive and Gram-negative microorganisms compared with the same metal-free porphyrins.

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REFERENCES

1. *Мадакян В.Н., Казарян Р.К., Хачатрян М.А., Степанян А.С., Куртикян Т.С., Ордян М.Б.* Новые производные мезо-тетра(4-пиридил)порфина и некоторые их превращения. Химия гетероциклических соединений, *2*, 212-216, 1986.
2. *Demidova T.N., Hamblin R.M.* Effect of cell-photosensitizer binding and cell density on microbial photoinactivation. Antimicrobial Agents and Chemotherapy, *49,* 6, 2329-2335, 2005.
3. *Gyulkhandanyan G.V., Ghazaryan R.K., Paronyan M.H., Gyulkhandanyan A.G., Sheyranyan M.A, Dzhagarov B.M., Tuchina E.S., Korchenova M.A., Tuchin V.V.* Photophysical properties and photodynamic efficiency of cationic porphyrins. Proc. of SPIE, 8942, 894212-1 - 894212-9, 2014.

4. *Gyulkhandanyan G.V., Paronyan M.H., Hovsepyan A.S., Ghazaryan R.K., Tovmasyan A.G., Gyulkhandanyan Aram G., Gyulkhandanyan Anna G., Amelyan G.V.* Photodynamic inactivation of Gram (-) and Gram (+) microorganisms by cationic porphyrins and metalloporphyrins. Proc. of SPIE, 7380, 73803I-1 - 73803I-7, 2009.
5. *Hamblin M.R., Hasan T.* Photodynamic therapy: a new antimicrobial approach to infectious disease? Photochem. Photobiol. Sci., *3*, 5, 436-450, 2004.
6. *Hamblin M.R., O`Donnell D.A., Mufthy N., Rajagopalan K., Michaud N., Sherwood M.E., Hasan T.* Polycationic photosensitizer conjugates: effect chain length and Gram classification on the photodynamic inactivation of bacteria. J. Antimicr. Chemotherapy, *49*, 941-951, 2002.
7. *Lambrechts S.A.G., Aalders M.C.G., Verbraak F.D., Lagerberg J.W.M., Dankert J.B., Schuitmaker J.J.* Effect of albumin on the photodynamic inactivation of microorganisms by a cationic porphyrin. J. Photochem. Photobiol. B:Biol., *79*, 51-57, 2005.
8. *Maisch T.* Anti-microbial photodynamic therapy: useful in the future? Lasers in Medical Science, *22*, 2, 83-91, 2007.
9. *Malik Z., Ladan H., Nitzan Y.* Photodynamic inactivation of Gram-negative bacteria: problems and possible solutions. J. Photochem. Photobiol. B., *14*, 262-266, 1992.
10. *Minnock A., Vernon D.I., Schofield J., Griffits J., Parish J.H., Brown S.B.* Photoinactivation of bacteria. Use of a cationic water-soluble zinc phthalocyanine to photoinactivate both Gram-negative and Gram-positive bacteria. J. Photochem. Photobiol. B: Biol., *32*, 159-164, 1996.
11. *Nitzan Y., Malik Z., and Eherenberg B.* Photosensitization of microbial cells. In Photobiology: the Science and its Applications, (Riklis E., Ed.), 815-820, Plenum Press, New York, 1991.
12. *Pashkovskaya A.A.,. Sokolenko E.A, Sokolov V.S., Kotova E.A., Antonenko Y.N.* Photodynamic activity and binding of sulfonated metallophthalocyanines to phospholipid membranes: Contribuition of metal-phosphate coordination. Biochim. Biophys. Acta, *1768*, 2459-2465, 2007.
13. *Tovmasyan A.G., Ghazaryan R.K., Sahakyan L., Gasparyan G., Babayan N., Gyulkhandanyan G.* Synthesis and anticancer activity of new water-soluble cationic metalloporphyrins. European Conferences on Biomedical Optics 2007, 17-21, June, 2007, Munich, Germany, Technical Abstract Summaries, 71-72, 2007.
14. *Wainwright M.* Photoantimicrobials – so what's stopping us? Photodiagn. Photodyn. Ther., *6*, 167-169, 2009.

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