Հայաստանի Գիտությունների Ազգային Ակադեմիա
Национальная Академия Наук Армении
National Academy of Sciences of Armenia

Հայաստանի Կենսաբանական Հանդես
Биологический Журнал Армении
Biological Journal of Armenia

• Փորձարարական և տեսական հոդվածներ • Экспериментальные и теоретические статьи •
• Experimental and theoretical articles •

Biolog. Journal of Armenia, 3 (64), 2012

INTERCELLULAR EFFECTS OF METAL CONTAINING MESO-TETRA-(4N-OXYETHYLPYRIDYL) PORPHYRINS: CYTOTOXICITY TO TWO PHOTON IMAGING

L.S. ABGARYAN, Y.B. DALYAN

Department of Molecular Physics, Yerevan State University,
lusinea@gmail.com

Porphyrins and metal-derivatives of porphyrins are one of the interesting investigation fields for biomedical research. Understanding the effects of porphyrins at the cellular level may promote development of novel methods of treatment of a series of disorders including cancer.

Two-Photon microscopy was performed in order to understand sub-cellular localization of investigated porphyrins. Furthermore the sub-cellular localizations of Ag, Co and Zn containing meso-tetra-(4N-oxyethylpyridyl) porphyrins (TOEPyPs) were correlated with their cytotoxic properties. In addition, the cytotoxicity of these compounds was also studied on wild type and mutated mammalian cell systems. It has been observed that even though at low concentrations all porphyrins are being equally distributed on cell surface, at high concentrations the Ag, Co and Zn containing porphyrins have dramatically different mode of interaction with mammalian cells: The two-photon microscopy revealed that only AgTOEPyP is being able to penetrate through the cell membrane and localize in particular organelles. Co and Zn containing TOEPyPs manly remain on cell surface and localizing in the cell membrane. Interestingly it was shown that AgTOEPyP is selectively cytotoxic for cells isolated from mammalian cancer nodules.

Porphyrin – mammalian cells – two photon microscopy

Պորֆիրինները, ինչպես նաև պորֆիրինների մետաղական ածանցիալները, մեծ հետաքրքրություն են ներկայացնում կենսաբժշկական հետազոտությունների համար: Բջջային մակարդակում պորֆիրինների ներգործությունը հասկանալու արդյունքում հնարավոր է զարգացնել նոր և առավել էֆեկտիվ բուժման մեթոդներ տարբեր հիվանդությունների համար՝ այդ թվում նաև քաղցկեղի:

Աշխատանքում օգտագործվել է երկֆոտոն միկրոսկոպիա, որպեսզի ուսումնասիրվի հետազոտվող պորֆիրինների տեղայնացումը բջիջներում: Ag, Co և Zn պարունակող meso-tetra-(4N-oxyethylpyridyl) պորֆիրինների (TOEPyPs) ներբջջային տեղայնացման էֆեկտները համակցվել են պորֆիրինների ցիտոտոքսիկ էֆեկտների հետ: Ավելին, պորֆիրինների ցիտոտոքսիկությունը ուսումնասիրվել է բնական և մուտացված (քաղցկեղային) բջիջների վրա: Ցույց է տրվել, որ, չնայած այն հանգամանքին, որ փոքր դոզաների դեպքում բոլոր երեք պորֆիրինները համասեռ բաշխվում են բջիջների մեմբրանի մակերևույթով, մեծ դոզաների դեպքում Ag, Co և Zn պարունակող պորֆիրինները տարբեր կերպ են փոխազդում բջիջների հետ: Երկֆոտոն միկրոսկոպիայի արդյունքները ցույց են տվել, որ AgTOEPyP թափանցում է բջջի մեջ և կուտակվում է որոշ օրգանելլաների մեջ: Co և Zn պարունակող TOEPy պորֆիրինները հիմնականում կուտակվում են բջջի մեմբրանային թաղանթի վրա: Նաև ցույց է տրվել, որ AgTOEPyP ընտրողաբար ցիտոտոքսիկ է գեղձերից անջատած քաղցկեղային բջիջների նկատմամբ:

Պորֆիրին – կաթնասունների բջիջներ – երկֆոտոն միկրոսկոպիա

Порфирины и их металло-производные являются интересными объектами для биомедицинских исследований. Понимание эффекта порфирина на клеточном уровне может помочь развитию новых методов лечения для ряда болезней, в том числе и рака.

Был применен метод двухфотонной микроскопии для того, чтобы понять локализации изучаемых порфиринов в клетках. Внутриклеточные локализации Ag, Co и Zn, содержащих meso-tetra-(4N-oxyethylpyridyl) порфиринов (TOEPyPs), были сопоставлены с их цитотоксическими эффектами. Более того, цитотоксичность этих порфиринов была изучена на натуральных и мутированных клеточных системах. Было показано, что несмотря на то, что в малых дозах все порфирины равномерно распределяются по поверхности клеток, а в больших дозах Ag, Co и Zn, содержащие порфирины, взаимодействуют с клетками по-разному. Результаты двухфотонной микроскопии показывают, что только AgTOEPyP проникает через мембрану клетки и локализуется в особых органеллах клетки. Co и Zn, содержащие TOEPy порфирины, в основном остаются и локализуются на поверхности клеточной мембраны. Обнаружено также, что AgTOEPyP селективным образом токсичен для раковых клеток, изолированных от узлов млекопитающих.

Порфирин – клетки млекопитающих – двухфотонная микроскопия

Porphyrins are a group of organic compounds, mostly naturally occurring, with the porphyn ring as a base of their structure. Porphyrins and porphyrin derivatives have an outstanding potential for therapeutics with their antimicrobial and antiviral properties, including anti-HIV (human immunodeficiency virus) activities [8-9, 11-13]. Many of them have potential to be used in the photodynamic therapy of tumors [2] because of being photosensitizers. Therefore porphyrins have been subjects of investigation for the past few years by our group [1, 3, 4, 10].

Numerous works has been conducted to elicit how these porphyrins interact with DNA. Within living systems, the penetration of these porphyrins into the cell supersedes their interaction. The ability of porphyrin to penetrate through cell membrane was assessed in the current work.

In this work the method of two photon microscopy was used in order to determine sites of interaction of AgTOEPyP4 with mammalian cells. The advantages of multiphoton microscopy include the localized excitation which allows for deep tissue and intracellular imaging. Each pixel obtained from the multiphoton XYZ scan can later be rendered into a full 3 dimensional image following by a series of post imaging analysis.

Materials and methods. The compounds studied were tetranitrate meso-tetra (4N-oxyethylpyridyl) porphynato Ag(II) (AgTOEPyP), Co(II) (CoTOEPyP), Zn(II) (ZnTOEPyP) (fig. 1). These agents are water-soluble, metal derivatives of TOEPyP synthesized by alkylation of meso-tetra (4-N-pyridyl) porphyn at the excess of ethylenchlorhydrine in dimethylformamide [5-7]. The molecular mass of AgTOEPyP, CoTOEPyP and ZnTOEPyP respectively are 1152 Da, 995.16 Da and 1000.15 Da. Variant concentrations of porphyrin were applied to a final amount of cells. The range of final concentrations of porphyrins was from 300 M to 0 M with 3 fold dilution and 8 concentration points.

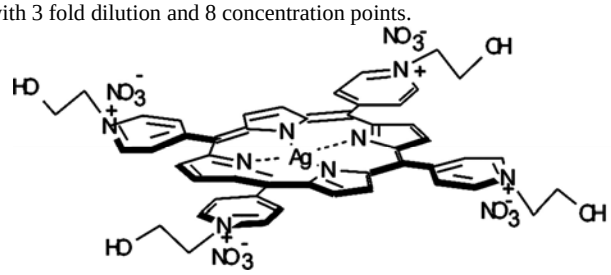

Fig. 1. AgTOEPyP porphyrin.

Immortalized murine bone marrow-derived pro-B-cells were used for this work. The cells were grown in DMEM (Dulbecco's Modified Eagle Medium) media containing 10 % FBS (fetal bovine serum), penicillin and streptomycin. Cell count during experiment was 60000 cells per por-

phyrin concentration or 300 ml of 200000 cells per dose of porphyrin. Trypan Blue has been used for calculating alive and dead cells after treatment.

Following porphyrin treatment, two-photon microscopy was conducted to aid in investigation of cell-porphyrin interaction based on porphyrins auto fluorescent property.

Results and Discussion. *Two Photon Microscopy*. Cells were treated with different doses of Porphyrin (300-0μM; 3 fold dilution) following by 2 days incubation in 37°C and 5% CO_2 incubator. After the 2 days treatment, the samples were imaged under two photon microscope.

In order to collect imaging data with the highest quality, emission peak of porphyrins needed to be determined. Therefore sample was excited by 880 nm wavelength femtosecond laser radiation and the detection signal was collected via a descanted mode. The detection spectrum was scanned from 586.17 nm to 721.91 nm range to determine the emission range (fig. 2). Emission peak of porphyrins was determined to be 660 nm. All samples were imaged with emission 660 nm.

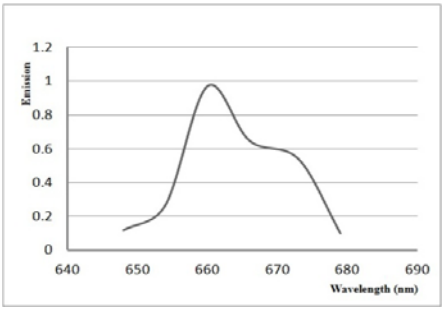

Fig. 2. Spectrum of porphyrins emission.

This experiment depicts the distribution of the porphyrins at low concentrations, showing the uniform distribution of the signal throughout the cell membrane.

With increasing concentration of AgTOEPyP, accumulation in localized areas within the cells starts to appear. As it is shown in fig. 3,it is apparent that Ag-porphyrin besides being distributed on entire cell surface, also have several highly localized regions inside the cells.

In fig. 4 single cell that was examined for more detail understanding of porphyrin localization in the cells, was presented from different angles (from 3D rendering). As one can see there are several dense porphyrin localization areas inside the cells, which we assume to be in nuclear and mitochondria.

For CoTOEPyP the localization is a little different. For low concentrations of CoTOEPyP the localization is the same. Porphyrin is manly interacting with cell membrane and being equally distributed through entire membrane. For higher concentrations, one can see on fig. 5, the cells treated with CoTOEPyP exhibit fluorescence localized within the cell membrane suggesting preferential interaction of this porphyrin with lipids of membranes. So chance that these porphyrins will penetrate through cell membrane and nuclear membrane to interact with DNA is really low.

The next porphyrin under investigation was ZnTOEPyP. At low concentration ZnTOEPyP is interacting equally with entire membrane surface. Cells treated with higher concentration of ZnTOEPyP exhibit fluorescence localized within the cell membrane suggesting preferential interaction of this porphyrin with preferential single area on membranes. Two photon image of mammalian cell treated with ZnTOEPyP is shown on fig.6.

Cytotoxicity

Within the scope of this work interaction of AgTOEPyP, CoTOEPyP and ZnTOEPyP with murine cells (healthy and cancer) has been investigated.

For cytotoxicity exam cells were treated with different doses of AgTOEPyP, CoTOEPyP and ZnTOEPyP. Cell count during experiment was 60000 cells per porphyrin concentration or 300 μl of 200000 cells per dose of porphyrin depending on cell adhesion profile. The range of concentration for each porphyrin studies was from 300μM to 0μM with 3 fold dilution and 8 concentration points.

For determining cytotoxicity effect of porphyrins on cells, after 2 days of incubation with ligand, cells were counted in complex with trypan blue to determine amount of alive and dead cells for each concentration of porphyrins.

Cytotoxicity test revealed that the AgTOEPyP and ZnTOEPyP porphyrins are selectively cytotoxic for cancer cells while CoTOEPyP exhibits no such selectivity (fig. 7).

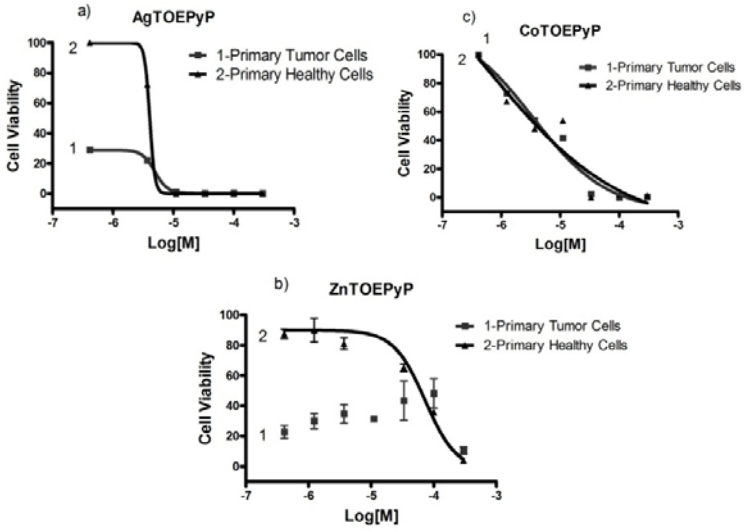

Fig. 7. Cytotoxicity effect of a) AgTOEPyP, b) CoTOEPyP and c) ZnTOEPyP on cells isolated from healthy animals and animals with cancer.

For all three porphyrins data is normalized so, that 100% cell viability corresponds to cells without porphyrin and 0% cell viability corresponds to the cells treated with the highest concentration (300 μM) porphyrin. Cytotoxicity analysis showed that AgTOEPyP has highly selective cytotoxic effect on cancer cells. ZnTOEPyP has a little less selectivity and CoTOEPyP does not have selectivity at all.

Current series of experiments utilizing this method revealed that the porphyrin under investigation has emission maximum at 660 nm. Upon determination of these optical properties the subsequent imaging was conducted at 660 nm. It has been observed that at low concentrations all three porphyrins interact manly with the cell membrane without considerably penetrations into the cytoplasm. Relatively high concentrations of AgTOEPyP result in infiltration into the cells and localization at particular organelles. Further analyses will determine the exact co-localization with cellular organelles. In order to show organelle localization of porphyrin signal, a specific cellular staining is required.

At high concentrations Co and Zn containing TOEPyPs remain on membrane and creating aggregation on specific areas.

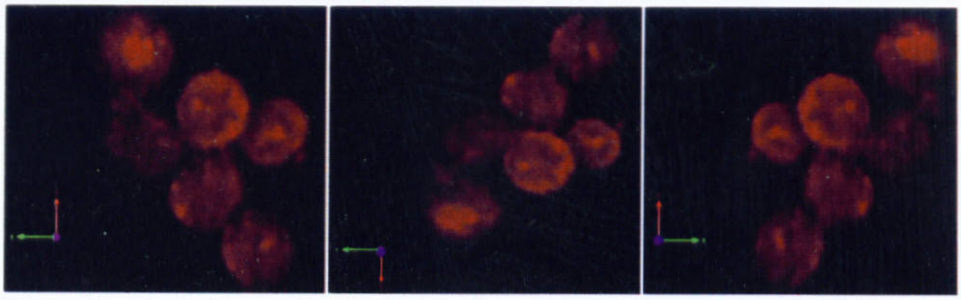

Figure 3

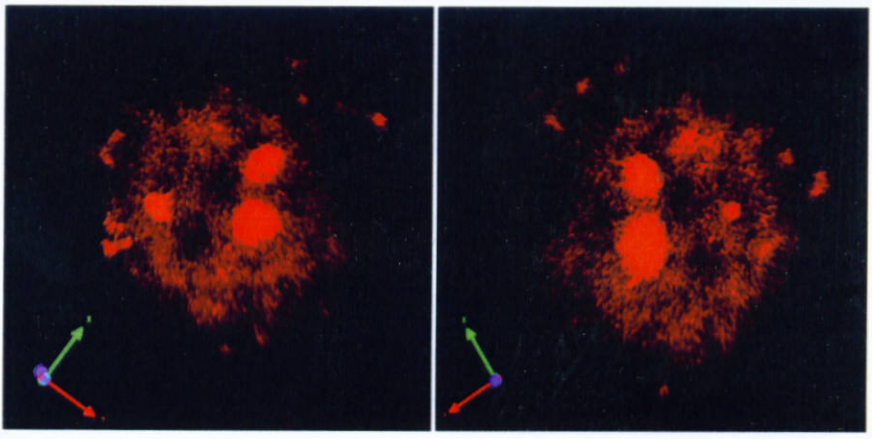

Figure 4

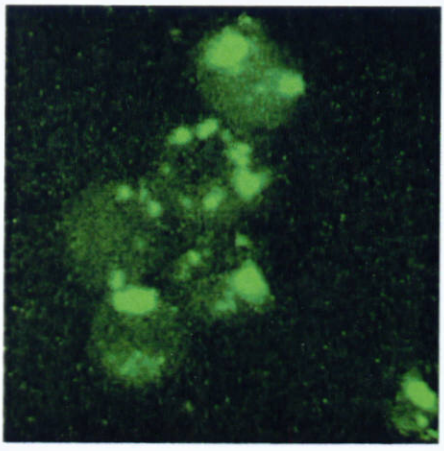

Figure 5

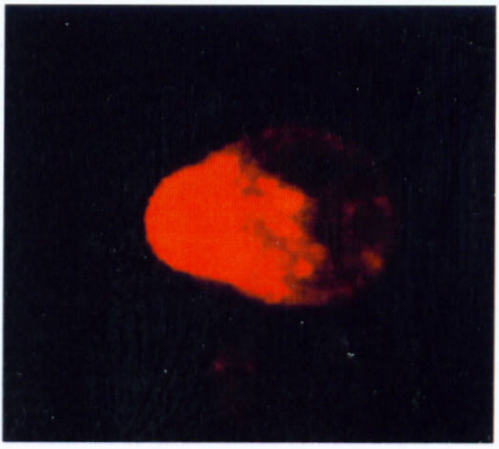

Figure 6

Cytotoxicity analysis showed that AgTOEPyP has highly selective cytotoxic effect on cancer cells. ZnTOEPyP has a little less selectivity and CoTOEPyP does not have selectivity at all.

Acknowledgments. We are grateful to Department of Genetics and Cytology of Yerevan State University, and Department of General and Organic Chemistry of Yerevan State Medical University and particularly to Prof. Rouben Aroutiounian, Prof. Gennadi Gasparyan, Prof. Robert Ghazaryan and Artak Tovmasyan for advice, valuable discussions, provided materials and information. This work was supported by the State Committee of Science of Armenia [11RB-017] and Belarusian Republican Foundation for Fundamental Research [X11Arm-013].

REFERENCES

1. *Dalyan Y.B, Haroutiunian S.G, Ananyan G.V, Vardanyan V.I, Lando D.Y, Madakyan V.N, Kazaryan R.K, Messory L, Orioli P, Benight A.S.* J Biomol. Struct. Dyn. Interactions of meso-tetra-(4-N-oxyethylpyridyl) porphyrin, its 3-N analog and their metallocomplexes with duplex DNA; *18*, 5, 677-87, 2001.
2. *Dougherty, T.J., C.J. Gomer, B.W. Henderson, G. Jori, D. Kessel, M. Korbelik, J. Moan, and Q. Peng.* Photodynamic therapy. J. Natl. Cancer I., *90*, 889–905, 1998.
3. *Ghazaryan A.A., Dalyan Y.B., Haroutiunian S.G., Vardanyan V.I., Ghazaryan R.K., Chalikian T.V.* Thermodynamics of interactions of TAlPyP4 and AgTAlPyP4 porphyrins with poly(rA)poly(rU) and poly(rI)poly(rC) duplexes. J Biomol Struct Dyn., *24*, 1, 67-74, 2006.
4. *Ghazaryan A.A., Dalyan Y.B., Haroutiunian S.G., Tikhomirova A, Taulier N., Wells J.W., Chalikian T.V.* Thermodynamics of interactions of water-soluble porphyrins with RNA duplexes. J Am Chem Soc. *15*, *128*, 6, 1914-21, 2006.
5. *Ghazaryan, R.K., L.A. Sahakyan, A.G.Tovmasyan, G.V. Gyulkhandanyan, and S.K. Yericyan.* Metallocomplexes of meso-tetra-pyridylporphyrins' derivatives with simultaneously fungicide and growth stimulating activities. Patent of the Republic of Armenia, N 1716 A2, 2006.
6. *Ghazaryan, R.K., L.A. Sahakyan, and A.G. Tovmasyan.* Derivatives of Meso-tetra-4-N-pyridylporphyne and their metallocomplexes. Patent of the Republic of Armenia, N 1714 A2, 2006.
7. *Ghazaryan, R.K., L.A. Sahakyan, A.G.Tovmasyan, and G.V. Gyulkhandanyan.* Silver complex of meso-tetra-(4-N-oxyethylpyridyl)porphyne possessing simultaneously fungicide and bactericide activities. Patent of the Republic of Armenia, N 1715 A2, 2006.
8. *Jori G.* Photodynamic therapy of microbial infections: state of the art and perspectives. J. Environ. Pathol. Toxicol. Oncol. *25*, 505–519, 2006.
9. *Lambrechts, S.A., M.C. Aalders, F.D.Verbraak, J.W. Lagerberg, J.B. Dankert, and J.J. Schuitmaker.* Effect of albumin on the photodynamic inactivation of microorganisms by a cationic porphyrin. J. Photochem. Photobiol.B 79:51–57 (2005).
10. *Monaselidze J, Majagaladze G, Barbakadze Sh, Khachidze D, Gorgoshidze M, Kalandadze Y, Haroutiunian S, Dalyan Y, Vardanyan V.* Microcalorimetric investigation of DNA, poly(dA)poly(dT) and poly[d(A-C)]poly[d(G-T)] melting in the presence of water soluble (meso tetra (4 N oxyethylpyridyl) porphyrin) and its Zn complex.; J Biomol Struct Dyn., *25*, 4, 419-24, 2008.
11. *Stojiljkovic, I., and G.G. Churchward.* Non-iron metalloporphyrins and methods of use. United States Patent, N 6066628, 2000.
12. *Stojiljkovic, I., B.D. Evavold, and V. Kumar.* Antimicrobial properties of porphyrins. Expert Opin. Inv. Drug., *10*, 309–320, 2001.
13. *Vzorov, A.N., D.W. Dixon, J. S. Trommel, L.G. Marzilli, and R.W. Compans.* Inactivation of human immunodeficiency virus type 1 by porphyrins. Antimicrob. Agents Chemother., *46*, 3917–3925, 2002.

Received 19.07.2012