

The Development of Zn (II) Porphyrins as a Photodynamic Inactivator of Antimicrobials

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Abstract

Tetra-cationic Zn (II) meso-tetrakis (N-alkylpyridinium-2 (or -3 or -4)-yl)Porphyrins (ZnPs) with progressively increased lipophilicity were synthesized to investigate how the tri-dimensional shape and lipophilicity of the photosensitizer (PS) affect cellular uptake, subcellular distribution, and photodynamic efficacy. The effect of the tri-dimensional shape of the molecule was studied by shifting the N-alkyl substituent attached to the pyridyl nitrogen from ortho to Meta and Para positions. Progressive increase of lipophilicity from shorter hydrophilic (methyl) to longer amphiphilic (hexyl) alkyl chains increased the phototoxicity of the ZnP PSs. PS efficacy was also increased for all derivatives when the alkyl substituents were shifted from ortho to meta, and from meta to Para positions. Both cellular uptake and subcellular distribution of the PSs were affected by the lipophilicity and the position of the alkyl chains on the periphery of the porphyrin ring. Whereas the hydrophilic ZnPs demonstrated mostly lysosomal distribution, the amphiphilic hexyl derivatives were associated with mitochondria, endoplasmic reticulum, and plasma membrane. A comparison of hexyl isomers revealed that cellular uptake and partition into membranes followed the order Para > Meta > ortho. Varying the position and length of the alkyl substituents affects (i) the exposure of cationic charges for electrostatic interactions with anionic biomolecules and (ii) the lipophilicity of the molecule. The charge, lipophilicity, and the tri-dimensional shape of the PS are the major factors that determine cellular uptake, subcellular distribution, and as a consequence, the phototoxicity of the PSs [1].

Keywords: Cell Proliferation; Fluorescence; Membrane Bilayer; Photodynamic Therapy; Porphyrin; Zn Porphyrin; Cellular Uptake; Photosensitizer; Phototoxicity; Subcellular Distribution

Introduction

Fanconi anemia (FA) is a genetically and phenotypically heterogeneous disorder characterized by congenital malformations, progressive marrow failure, and a marked predisposition to malignancy. Hematologic abnormalities occur in FA patients at a median of 7 years (range: birth to 31 years). By 40 years of age, the risk of developing bone marrow failure is 90%, advanced myelodysplastic syndrome (MDS). Or acute leukemia 33%, and no hematologic malignancies 28%. To date, allogeneic hematopoietic cell transplantation (HCT) remains the only proven therapy to potentially cure the hematologic abnormalities of FA.

Photodynamic therapy (PDT) is a relatively non-invasive therapeutic option for treating neoplastic and non-neoplastic diseases. Approved initially for the treatment of a small number of selected tumours, it has expanded to encompass a wide range of applications in dermatology, ophthalmology, dentistry, cardiology cosmetics, blood purification and water disinfection [2].

PDT is based on the preferential uptake of a photosensitive dye, the photosensitizer (PS), by the targeted cells/tissue, followed by irradiation of the selected area with visible light. Upon absorption of light the PS reaches an excited state, which is followed by either transfer of electron or abstraction of hydrogen atom (type I reaction) to/from a neighboring organic molecule or transfer of energy or electron to oxygen (type II reaction) to generate singlet oxygen (1O_2) and superoxide radical (O_2^-). As a result of these reactions, biomolecules and cellular structures could be modified to an extent that causes cell death. Because most of the species generated by the excited PS have a short life in the cellular environment, the range of immediate damage is limited by the localization of the PS. The nature of the damaged structures and the extent of damage determine the predominant mechanism of cell death, which in turn is a major predictor of PDT

outcome. It has been shown that overall charge, charge distribution, and lipophilicity of the molecule are among the most important parameters that control cellular uptake and subcellular distribution of a PS. Our previous investigations with Zn porphyrin-based PSs and photo inactive Mn porphyrin analogs revealed that an additional factor influencing the uptake and localization of cationic molecules is the nature of the substituents linked at the periphery of the porphyrin ring; these substituents determine the size, three-dimensional shape, and the bulkiness of the molecule. Initial experiments showed that fluorescence of a number of Zn alkylpyridylporphyrin derivatives was sufficient for assessment of cellular and subcellular localization by fluorescence microscopy [3].

Material and Methods

Cell Culture

The LS174T human colon adenocarcinoma cell line was used in this study. To check for cell line-specific effects, the MCF7 breast cancer cell line was used in parallel. Monolayer cultures were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum, 1% l-glutamine, 1% penicillin/streptomycin as an antibacterial agent and 0.1% amphotericin as an antifungal agent. Cultures were maintained at 37°C and 5% CO₂ and used for experiments at 80–90% confluence. The growth medium was removed, and the cells were washed with

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phosphate-buffered saline (PBS). Cells were then detached by trypsinization and incubation at 37 °C for 2–3 min. Overexposure to trypsin was avoided because excessive trypsinization results in leaky and nonviable cells. Fresh medium (10× trypsin volume) was added to inhibit trypsin action. The cell suspension was centrifuged at 500 × g for 3 min. The supernatant was discarded, and the pellet obtained was resuspended in fresh medium. Cells were then counted and used in experiments, and the remaining portion was subcultured and maintained. Cells were counted prior to seeding into the plates or use in experiments. Cell counting was performed with an improved Neubauer hemocytometer and trypan blue to differentiate between viable and nonviable cells [4].

Photosensitizers

Photosensitizers investigated in this study were isomeric methyl (ZnTMPyP), ethyl (ZnTEPyP), butyl (ZnBuPyP), and hexyl (ZnHxPyP) (Fig. 1) Zn (II) N-alkylpyridylporphyrins. Stock solutions were prepared in distilled water and filter-sterilized.

Light Source

Cell cultures were illuminated by two 5-W, white fluorescent light tubes mounted under a translucent screen providing a fluence of 2.0 mW/cm².

Phototoxicity of Zn (II) N-Alkylpyridylporphyrins

The photoefficacy of the Zn (II) N-alkylpyridylporphyrins was assessed by investigating effects on cell viability and cell proliferation [5].

Cell Viability

Cell viability was determined by the surrogate MTT assay based on metabolic reduction of the yellow tetrazolium dye MTT to an insoluble purple-colored formazan product that can be determined spectrophotometrically (24). The cells were counted, seeded into a flat-bottom 96-well microplate at a concentration of 5 × 10⁴ cells in 100 µl of medium/well and incubated overnight to permit cells to adhere. ZnPs were added at the indicated concentrations in quadruplicate wells, and cells were incubated with the PSs in the dark for 6, 12, and 24 h. Control wells without PSs were incubated under the same conditions. After the incubation period, the medium was replaced with PBS, and plates were subjected to illumination (or dark incubation). Following the illumination, PBS was removed and replaced with culture medium. The MTT reagent (10 µl/well), prepared by dissolving 5 mg of MTT in 1 ml of PBS, was then added to each well. The cultures were incubated for 3 h at 37 °C, and then 10% SDS in 0.01 M HCl was added followed by incubation overnight. Absorbance was measured at 560 (formazan product) and 650 nm (background) using a microplate reader. Dark toxicity of the tested compounds was assessed by following the same protocol except that illumination was omitted [6].

Cell Proliferation

Cell proliferation was followed using the sulforhodamine B (SRB) assay (25), which is based on binding of SRB to proteins of cells fixed with trichloroacetic acid (TCA). The cells were counted and seeded into a flat-bottom 96-well microplate at 5 × 10³ cells/well and were left overnight to adhere. ZnPs were added to quintuplet wells, and cells were incubated in the dark for 24 h. Control wells had no PS added. After the incubation, the medium was replaced with PBS, and the plates were illuminated. After illumination, PBS was replaced with medium. The SRB assay was performed at 0, 24, 48, and 72 h, with zero time

being immediately after illumination. Cells were fixed with cold TCA to a final concentration of 10%. The plates were then incubated at 4 °C for 1 h, followed by five washes with deionized water. Fixed cells were stained with 0.4% SRB dissolved in 1% acetic acid for 30 min. Cells were then washed five times with 1% acetic acid to remove unbound stain and air-dried at room temperature. The bound dye was solubilized with 10 mM Tris solution with volume equal to the volume of the original culture medium, and the content of the wells was mixed and left for 5 min. The plates were analysed on a microplate reader at 510 nm, and the background was measured at 690 nm [7].

Cellular Uptake of Zn (II) N-Alkylpyridylporphyrins

Cells were counted and seeded in a 6-well plate at 25 × 10⁴ cells/well and were incubated to ~80% confluence. ZnP isomers were added at a concentration of 20 µM, and the cells were incubated with the PSs in the dark for 24 h. The medium was then removed, and the cells were washed with PBS and solubilized with 0.25% (v/v) Brij-98. The intracellular accumulation of ZnPs was assayed by measuring their fluorescence emission at an excitation wavelength (λ_{ex}) matching their Soret band and emission wavelength range (λ_{em}) of 520–700 nm. Peak areas at emission were calculated, and the amount of ZnP in each sample was determined using peak areas generated by pure Zn (II) P isomers with known concentrations as standards. Quinine was used as a fluorescence standard.

Discussion

Singlet oxygen is regarded as the principal damaging species in photodynamic treatment. Its short lifetime (~4 µs in pure water) implies that the maximum distance it can travel (assuming no reaction with any biomolecule occurs) would be ~125 nm. Taking into account the average size of mammalian cells (~10–30 µm) or even of organelles such as mitochondria (~500 nm) it becomes clear that the primary reaction targets of ¹O₂ would be determined by PS localization. Uptake and subcellular localization of PSs in turn are controlled mainly by the net charge and lipophilicity of the molecule. Studies on photosensitive MnP analogs have shown that desired lipophilicity can be achieved by adding suitable alkyl substituents at the meso pyridyl positions, without affecting the net positive charge of the molecule. Assessment of partitioning between n-octanol and water revealed that each additional carbon atom added to the N-alkyl chains increases lipophilicity by 10-fold. An additional 10-fold increase of lipophilicity can be achieved by moving the N-alkyl groups from the ortho to Meta N-pyridyl position. Here we demonstrate that a 50-fold increase in the lipophilicity of Zn N-alkylpyridylporphyrins produced by lengthening the side chains from 1 (methyl) to 6 carbons (hexyl), caused ~5-fold increase in the uptake of the PS by the cancer cells. As a result, the amphiphilic hexyl derivatives (ZnTnHexPyP) were more efficient than the more hydrophilic ZnTMPyP derivatives in suppressing cell metabolism (MTT assay) and cell proliferation. In addition, our study disclosed significant differences among the ortho, Meta, and Para isomers with respect to the photo efficiency [8].

Knowledge obtained with fluorescent Zn porphyrin photosensitizers will help in the development of related families of alkylmetalporphyrins with similar structures but different activities. The Zn Porphyrins act as potent photosensitizers, but replacement of the chelated metal gives compounds capable of rapid redox cycling, superoxide dismutase-mimetic function, and compounds that show promise as fluorescent imaging agents. Compounds with very similar or identical molecular dimensions and very similar physical properties, e.g. lipophilicity, will show strong similarities in cellular and subcellular

accumulation and distribution. Study of localized photochemical modification actions of these Zn analogs thus generates a body of knowledge of much broader significance with a wide potential range of applications for investigation and manipulation of cell responses and signal pathways, including induction of apoptotic and necrotic cytotoxic responses, in scientific investigations and future therapeutic applications [9].

Conclusion

The management of HCC has changed substantially in the past few decades based on recent advances in the understanding of HCC pathophysiology and the development of new therapies. Based on new molecular knowledge and recognition of the limitations of sorafenib, novel molecularly targeted therapies and combination strategies have been developed. Although early phase data with these agents have looked promising, to date nothing has been shown to be better than sorafenib. Incorporating these new agents as an adjuvant therapy for TACE provides an opportunity to increase our understanding of these agents in HCC. Moreover, inhibition of hypoxia-induced survival signals might be additionally required as adjuvant therapy following TACE or anti-antigenic therapies commonly result in significant tumor hypoxia. Therefore, further basic experiments and clinical studies are required to enhance the therapeutic potency of TACE in the treatment of HCC [10].

Conflicts of interest

None.

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References

1. Tyagarajan S, Schmitt D, Acker C (2019) Autologous cryopreserved leukapheresis cellular material for chimeric antigen receptor-T cell manufacture. *Cytotherapy* 21: 1198-1205.
2. McGuirk J, Waller EK, Qayed M, Abhyankar S, Ericson S, et al. (2017) Building blocks for institutional preparation of CTL019 delivery. *Cytotherapy* 19: 1015-1024.
3. Qayed M, McGuirk JP, Myers GD, Parameswaran V, Waller EK, et al. (2022) Leukapheresis guidance and best practices for optimal chimeric antigen receptor T-cell manufacturing. *Cytotherapy* 24: 869-878.
4. Zhu F, Shah N, Xu H, Schneider D, Orentas R, et al. (2018) Closed-system manufacturing of CD19 and dual-targeted CD20/19 chimeric antigen receptor T cells using the CliniMACS Prodigy device at an academic medical center. *Cytotherapy* 20: 394-406.
5. Xu J, Melenhorst JJ, Fraietta JA (2018) Toward precision manufacturing of immunogene T-cell therapies. *Cytotherapy* 20(5):623-638.
6. Gill S, June CH (2015) Going viral: chimeric antigen receptor T-cell therapy for hematological malignancies. *Immunol Rev* 263: 68-89.
7. Sur D, Havasi A, Cainap C, Samasca G, Burz C, et al. (2020) Chimeric Antigen Receptor T-Cell Therapy for Colorectal Cancer. *J Clin Med* 9: 182.
8. Schneider D, Xiong Y, Wu D, Nölle V, Schmitz S, et al. (2017) A tandem CD19/CD20 CAR lentiviral vector drives on-target and off-target antigen modulation in leukemia cell lines. *J Immunother Cancer*. 5: 42.
9. Zhu Y, Tan Y, Ou R, Zhong Q, Zheng L, et al. (2016) Anti-CD19 chimeric antigen receptor-modified T cells for B-cell malignancies: a systematic review of efficacy and safety in clinical trials. *Eur J Haematol* 96:389-396.
10. Maus MV, Levine BL (2016) Chimeric Antigen Receptor T-Cell Therapy for the Community Oncologist. *Oncologist* 21: 608-617.