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Review

Tetra-triethyleneoxysulfonyl substituted zinc phthalocyanine for photodynamic cancer therapy

Weronika Kuzyniak[a], Eugeny A. Ermilov[a,d], Devrim Atilla[b], Ayşe Gül Gürek[b], Bianca Nitzsche[a], Katja Derkow[c], Björn Hoffmann[a], Gustav Steinemann[a], Vefa Ahsen[b], Michael Höpfner[a,*]

[a] Institute of Physiology, Charité-Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany
[b] Department of Chemistry, Gebze Institute of Technology, PO Box 141, Gebze, 41400, Turkey
[c] Department of Neurology, Charité-Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany
[d] Institute of Physics, Humboldt-Universität zu Berlin, Newtonstr. 15, 12489 Berlin, Germany

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ABSTRACT

Photodynamic therapy (PDT) has emerged as an effective and minimally invasive treatment option for several diseases, including some forms of cancer. However, several drawbacks of the approved photosensitizers (PS), such as insufficient light absorption at therapeutically relevant wavelengths hampered the clinical effectiveness of PDT. Phthalocyanines (Pc) are interesting PS-candidates with a strong light absorption in the favourable red spectral region and a high quantum yield of cancer cell destroying singlet oxygen generation. Here, we evaluated the suitability of tetra-triethyleneoxysulfonyl substituted zinc phthalocyanine (ZnPc) as novel PS for PDT.

ZnPc-induced phototoxicity, induction of apoptosis as well as cell cycle arresting effects was studied in the human gastrointestinal cancer cell lines of different origin. Photoactivation of ZnPc-pretreated (1–10 μM) cancer cells was achieved by illumination with a broad band white light source (400–700 nm) at a power density of 10 J/cm^2.

Photoactivation of ZnPc-loaded cells revealed strong phototoxic effects, leading to a dose-dependent decrease of cancer cell proliferation of up to almost 100%, the induction of apoptosis and a G1-phase arrest of the cell cycle, which was associated with decrease in cyclin D1 expression. By contrast, ZnPc-treatment without illumination did not induce any cytotoxicity, apoptosis, cell cycle arrest or decreased cell growth. Antiangiogenic effects of ZnPc-PDT were investigated in vivo by performing CAM assays, which revealed a marked degradation of blood vessels and the capillary plexus of the chorioallantoic membrane of fertilized chicken eggs. Based on our data we think that ZnPc may be a promising novel photosensitizer for innovative PDT.

Contents

* Corresponding author. Fax: +49 30 450 528 918.
E-mail addresses: weronika.kuzyniak@charite.de (W. Kuzyniak), ermilov@physik.hu-berlin.de (E.A. Ermilov), datilla@gyte.edu.tr (D. Atilla), gurek@gyte.edu.tr (A.G. Gürek), Bianca.Nitzsche@charite.de (B. Nitzsche), katja.derkow@charite.de (K. Derkow), Bjoern.Hoffmann@charite.de (B. Hoffmann), gustav.steinemann@charite.de (G. Steinemann), ahsen@gyte.edu.tr (V. Ahsen), michael.hoepfner@charite.de (M. Höpfner).

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1. Introduction

The therapeutic effect of light has been known for thousands of years in China or ancient Egypt. Only in nineteenth century photodynamic therapy (PDT) has been introduced to modern medicine by Herman von Tappeiner and Albert Jesionek, who treated skin cancer by combining a photoactive agent and light [1]. In 1977, PDT was introduced in clinical studies and then approved for cancer treatment in several countries (e.g. United States, Canada, Russia and Germany) [2,3].

PDT is a cancer therapy modality that involves the use of light at specially selected wavelengths to selectively excite a photosensitizer of choice in the presence of molecular oxygen. The activation of the PS results in the generation of reactive oxygen species (ROS) which damage and kill the target cells [4]. The advantages of PDT treatment over conventional cancer therapies are repeatability, excellent cosmetic effects (e.g. skin lesion treatment) and minor pain during and after treatment [5–7]. Nonetheless, increase of survival ratio and probability of long-term local disease control make PDT extremely attractive itself or as complement to other therapies [8].

However, several side effects of the PSs that have been approved for PDT treatment so far, limit the clinical applicability. Still, the main problems include long term photosensitivity and poor light absorption of PS [9,10]. Hence, there is need for new, better photoactive compounds. Phthalocyanines (Pcs) belong to the advanced group of so called second generation photosensitizers, which gather most attention for PDT nowadays [11,12]. Pcs are organic macrocyclic compounds which are similar in structure to naturally occurring porphyrins. Pcs are photostable, have a strong absorption in the red spectral region (with maximum at ca. 680–700 nm), which is regarded as the "therapeutic window" for effective PDT, and show a high quantum yield of singlet oxygen generation. Upon illumination the Pcs cause cell death *via* necrosis and – even more important – also via induction of apoptosis [13,14]. The substitution of alkylated polyethyleneglycol moieties to Pc [15–17] increased the solubility of the phthalocyanines in highly polar solvents and water. Thus highly solubility properties of these compounds provide convenience for in vitro studies.

Only recently, we reported on the synthesis, and the photochemical and photophysical properties of tetra-triethyleneoxysulfonyl substituted zinc phthalocyanine [15]. Preliminary in vitro studies have shown that ZnPc applied to human breast cancer cells resulted in photoinduced cytotoxicity at low light doses of 5 mW/cm^2 [15]. The major goal of the present study was to investigate the effectiveness of ZnPc PDT and to characterize the underlying mode of action of the treatment in terms of apoptosis induction, cell cycle effects, as well as general cytotoxicity and antiproliferative potency. The effects were confirmed on the in vivo level by performing CAM assays to additionally monitor antiangiogenic effects of the ZnPcs.

2. Material and methods

2.1. Compound

Tetra-triethyleneoxysulfonyl substituted zinc phthalocyanine (ZnPc) was prepared by slightly modifying procedure described in literature [15]. The synthetic pathway of the preparation of ZnPc and its structure is shown in Scheme 1.

Synthesis: 4(4,7,10-Trioxaundecan-1-sulfonyl) phthalonitrile [18] (0.30 g, 0.98 mmol) and anhydrous Zn(OAc) 2 (92 mg, 0.50 mmol) were refluxed in *N*,*N*′-dimethylaminoethanol (2 ml) for 24 h under argon atmosphere. *N*,*N*′-dimethylaminoethanolwas distillated off and the reaction mixture was diluted with dichloromethane. The product was precipitated from dichloromethane in hot hexane and filtered. The green waxy crude product was purified over a silica gel column using chloroform–ethanol mixture (15:1) as eluent (139 mg, 44%). $C_{60}H_{72}N_8O_{12}S_4Zn$, MW 1288.34; found C, 56.85; H, 5.26; N, 8.53; requires C, 56.12; H, 5.65; N, 8.72 ^{1}H NMR: (DMSO-d_6) δ 8.87 (d, 4H, aromatics), 8.82 (s, 4H, aromatics), 8.04 (d, 4H, aromatics), 4.04 (t, 8H, CH_2), 3.78 (t, 8H, CH_2), 3.70 (t, 8H, CH_2), 3.67 (t, 8H, CH_2) 3.57 (t, 8H, CH_2), 3.48 (m, 8H, CH2) 3.42 (t, 8H, CH_2), 3.22 (s, 12H, CH_2). MS (ESI): an isotopic pattern peaking at *m/z* 1289.3 [98%, $(M+H)^+$], 1291.3 [100%, $(M+3H)^+$], 1293.3 [75%], 1294.3 [42%], 1295.3 [23%], 1296.3 [7%].

The compound was dissolved in DMSO of spectroscopic grade (Aldrich) which was used without further purification. Concentration of stock solutions was calculated by measuring their optical density at 694 nm with UV–vis spectrometer Ultraspec 2100 (Amersham Biosciences) and using Lambert–Beer relationship with $\epsilon_{694nm} = 2.04 \times 10^5\ M^{-1}\ cm^{-1}$ [15]. Fluorescence excitation and emission spectra, were recorded on a Varian Eclipse spectrofluoremeter using 1 cm pathlength cuvettes at room temperature in DMSO (Fig. 1).

2.2. Cell culture

Human pancreatic carcinoid BON cells [19] were cultured in DMEM/Ham's F-12 (1:1) medium (Biochrom AG) supplemented with 10% fetal calf serum (FCS, Biochrom), 100 U/ml penicillin and 100 μg/ml streptomycin (Biochrom AG). The human esophageal squamous carcinoma cell lines Kyse-70 and Kyse-140 [20] were cultured in RPMI 1640 medium (Biochrom AG) supplemented with 10% fetal bovine serum (FCS, Biochrom), 100 U/ml