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Research Paper

THE BIOACTIVITY OF PHTHALOCYANINE DERIVATIVE FOR PHOTODYNAMIC THERAPY

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Abstract

Phthalocyanine derivatives (Pcs) are commercially applied as photosensitizers (PSs) in the technical and medical areas on a large scale, and more specifically in photodynamic therapy (PDT) of cancer. These derivatives (symmetrical and unsymmetrical substituted) have been found to be of great interest due to their distinctive characteristics, and several attempts have been made to modify them. Accordingly, here in a novel unsymmetrically substituted A3B type zinc (II) phthalocyanine photosensitizers (ZnPc-c) were synthesized through a multistep reaction and assessed. This compound was characterized using elemental analyses such as ultraviolet-visible spectroscopy (UV-vis) technique. In addition, their Suitability was evaluated as a novel PS for PDT. The ZnPc-c induced phototoxicity, induction of apoptosis as well as cell cycle arresting effects were studied in the mice cancer cell lines of different origins. The new compounds were detected and their antitumor activity in vivo and in vitro was also investigated. It was observed that ZnPc-c displayed a characteristic long-wavelength absorption peak at 670 nm. Moreover, the growth of Eca-109 cells in BALB/c nude mice was significantly inhibited in vivo. Clearly, ZnPc-c tends to have an excellent anti-tumor effect in vitro and in vivo, which could be considered as a potential photosensitizer of PDT in tumor treatment.

Key words: Photodynamic Therapy (PDT), zinc phthalocyanine (ZnPc), Photosensitizer (PS), Anti-tumor and Phthalocyanine derivative.

INTRODUCTION

The conventional cancer treatment options such as chemotherapy, radiotherapy, and surgery are not effective enough to remove all the malignant cells, and adversely affects healthy tissues and organs. Therefore, there is an urgent need for developing accessible

and affordable cancer treatment modalities. One of the treatments developed recently is Photodynamic therapy (PDT). PDT essentially consists of three major components: photosensitizer (PS) responsible for sensitivity of diseased tissue, light of specific wavelength causing mobilization of PS and oxygen. PDT, a promising alternative to the existing cancer therapies utilizes PS, is affordable, and does not require hospitalization of the patient [1]. Phthalocyanine derivatives (Pcs) can be applied as PS.

Phthalocyanine (Pc) is attracting much interest due to its characteristic 18π-electron delocalization in planar conjugated system, and its ability to chelate metal ions at the center of the ring, as well as the outstanding electronic and unconventional physical properties, high chemical and thermal stability. It has various applications in different technological and medical areas such as chemical sensors [1], electrochromic displaying systems [2], non-linear optics [3], solar cells [4, 5], molecular electronics [6], semiconductors [7], liquid crystals [8], catalyst [9] and photodynamic therapy (PDT). Furthermore, it has been extensively used as dyes and pigments [10] in the industries due to its blue-green color. However, the separation of unsymmetrically substituted A3B-type Pcs from others byproduct is much difficult due to similar chemical structures. Compared to unsymmetrical phthalocyanines, the yield of symmetrical phthalocyanines is very high. Consequently, recently, specific focus is to increase the percentage yield of symmetrical phthalocyanines. Phthalocyanines are poorly soluble in the water hence continuous efforts to design and synthesize more efficient water-soluble and NIR absorbing PS.

In this context, the chemical characterization, photophysical properties, and photodynamic activities in vitro and in vivo of ZnPc-c are reported.

2. MATERIALS AND METHODS

All solvents and reagents were obtained from commercial suppliers and used without further purification unless otherwise stated. The solvent was removed by rotary evaporation below reduced pressure. UV-vis absorption spectrum was recorded on an ultraviolet-visible spectrophotometer (Model V-530, Japan). Fluorescence spectra were measured on a fluorescence spectrophotometer (FluoroMax-4, France).

2.1. UV-visible Absorption and emission spectra

The UV-visible absorption spectrum was recorded on an ultraviolet-visible spectrophotometer. Fluorescence spectrum was measured on a Fluorescence

Spectrophotometer. Slits were kept narrow to 1 nm in excitation and 2 nm in emission. All the measurements were carried out at room temperature in quartz cuvettes with a path length of 1 cm. The compound was dissolved in DMSO, then it was diluted in different concentrations and measured it.

2.2.Cell line and culture conditions

Eca-109 cells were obtained from the Type Culture Collection of the Chinese Academy of Sciences. All cell culture related reagents were purchased from Shanghai Ming Rong BioScience Technology Co., Ltd. Cells were cultured in a normal RPMI-1640 culture medium. All media were supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin G and 100μg/mL streptomycin. All cells were incubated at 37℃ in 5% CO2 in a humidified incubator.

2.3. MTT cell viability assay

The Eca-109 cells were harvested and seeded in 96-well plates at 2×104 cells per well. The cells were allowed to attach to the bottom of the wells for 24 hours before starting the experiment at 5% CO2 at 37℃. ZnPc-c was administered to cells and allowed to uptake for 24 hours to test dark toxicity. Then RPMI-1640 medium containing drugs was removed and cells were washed with fresh PBS before irradiation with different light doses (ranging from 1 to 6 J cm-2) using an Nd: YAG laser at 670 nm when the phototoxicity was tested at 4 μM. The cell viability was evaluated by MTT colorimetric assay 24 hours after treatment.

2.4. In vivo PDT efficacy

ZnPc-c were injected to five-week-old BALB/c nude mice bearing Eca-109 tumors via the lateral tail vein at a dose of 5mg/kg in 0.2 mL solution. PDT was performed following injection with laser light (670 nm, 180 J/cm2,150 mW/cm2). Visible tumors were measured using two orthogonal measurements L and W (perpendicular to L), and the volumes were calculated using the formula V = LW2/2 and recorded.

3. RESULTS

3.1. UV–Vis absorption and fluorescence spectra

ZnPc-c displayed photosensitivity characteristic Soret and Q-band absorptions at 345 nm (Soret), and 676 nm (Q-band), respectively (Figure 1a). Meanwhile, when excited at 676 nm, ZnPc-c showed the strong emission peak at 680 nm (Figure 1b).

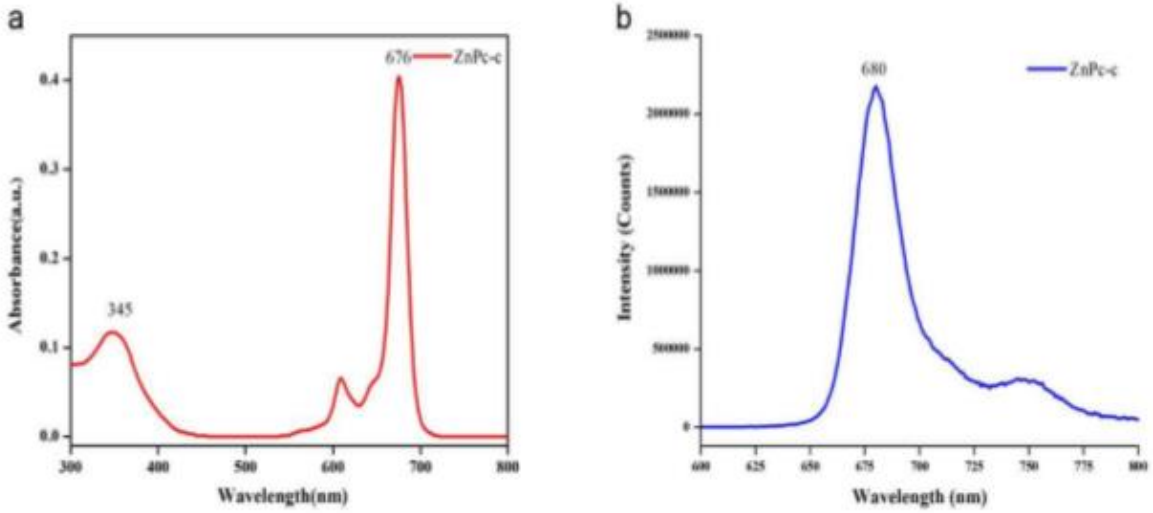

Figure 1. (a) UV-visible absorption spectrum of ZnPc-c in DMSO. **(b)** Fluorescence spectrum of ZnPc-c in DMSO at 2 μM.

3.2. Cytotoxicity on Eca-109 cells

The effect of phthalocyanine derivative on the viability of cultured Eca-109 cells was evaluated by MTT assay. The results indicated that the ZnPc-c had medicinal properties with low dark toxicity and high phototoxicity (Figure 2).

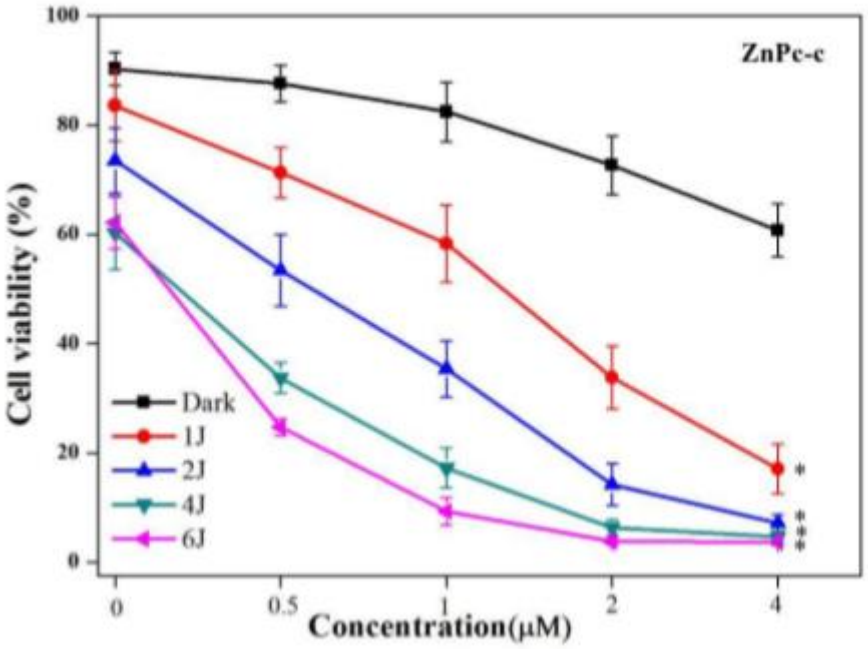

Figure 2. Cytotoxicity of ZnPc-c on Eca-109 cells.

3.3. In vivo photodynamic antitumor potency

The PDT anti-tumor efficacy of ZnPc-c was evaluated in Eca-109 tumor-bearing BALB/c nude mice. By comparing the tumor weight in different groups, the inhibition rates of tumor growth could be calculated. When tumor sizes had reached 6-8 mm in diameter, the mice were given intravenous injection via tail vein at a dose of 0.5 mg/kg. After 4 h incubation, the tumor site was irradiated with laser light (670 nm, 180 J/cm2, 150 mW/cm2). After five days of administration, treatment effects became significant. The volume of tumors in three sets of parallel control groups was larger than that in Laser+ ZnPc-c group. The tumor volume increased by 10 folds for 14 days in the control group.

4. DISCUSSION

In this paper, we show how photodynamic treatments affect Eca-109 tumor cells with ZnPc-c. The photodynamic activities were evaluated in vitro and in vivo. The ZnPc-c played a characteristic long-wavelength absorption peak at 676 nm. ZnPc-c induces no low dark toxicity and high photo-toxicity in the range of concentrations used in the present photodynamic studies

in vitro. In vivo therapeutic efficacy of PDT by using ZnPc-c, after being exposed to 670 nm laser light irradiation, the growth of Eca-109 tumor cells in BALB/c nude mice was significantly inhibited. Therefore, ZnPc-c is a promising antitumor photosensitizer for photodynamic therapy.

ACKNOWLEDGEMENT

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