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Photodynamic efficiency of a chlorophyll-a derivative in vitro and in vivo

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ABSTRACT

This paper reports the antitumor activity of a chlorophyll-a derivative, 2-[1-hydroxyethyl]-2-devinylpyropheophorbide-a (HEPa). Photophysical characteristics of HEPa were measured. And its cytotoxicity, intracellular localization, biodistribution, efficiency of photodynamic therapy (PDT), histological analysis were investigated using human bile duct carcinoma cells (QBC-939) and QBC-939 tumor bearing BABL/c nude mice as animal model. The results showed that HEPa was localized mainly within the cytoplasmic region and partially in lysosome. Biodistribution of HEPa in QBC-939 tumor bearing BABL/c nude mice showed its fast rate of clearance and high tumor selectivity. In vitro, HEPa had low dark toxicity and high photoxicity against QBC-939 cells. The inhibition rate of QBC-939 tumor could increase up to 92.3%, and H&E staining confirmed that HEPa could cause serious damage to the tumor with light dose of 100 J/cm^2, implying that HEPa has potential to be a new antitumor candidate for photodynamic therapy (PDT).

1. Introduction

Photodynamic therapy (PDT) has emerged as a promising therapeutic for cancers, which consists of the administration of a photoactivatable compound (photosensitizer, PS) and subsequent exposure of the target diseased tissue to light of appropriate wavelength in the presence of tissue oxygen [1–3]. In contrast to surgery, radiotherapy and chemotherapy, PDT is minimally invasive, local targeting, and has few systemic side effects.

The activation of PS upon light illumination results in generation of highly reactive oxygen species (ROS), for example singlet oxygen ($^1O_2$), ROS then attacks cellular targets, causes destruction through direct cellular damage, vascular shutdown, and activation of an immune response against targeted cells [4,5]. Tumors were destroyed by PDT though multifactorial mechanisms: (1) direct effect on the tumor cells, producing cell death by necrosis and/or apoptosis, (2) effect on the tumor vasculature, shutdown the vessels and depriving the tumor of oxygen and nutrients, (3) effect on the immune system, induction of a local inflammatory reaction [6–9].

The efficiency of a photosensitizer is one of the main factors for determining the feasibility of PDT. In recent years, many novel photosensitizers were synthesized and their biological activity was investigated. However, only a few photosensitizers have been proved for clinical use. For example, Photofrin (HpD) is the first photosensitizer to receive regulatory approval for the treatment of various cancers [10]. However, HpD is a mixture of at least types of nine components, causes cutaneous photosensitivity and has a weak absorption in the “optical window” [11,12].

To overcome the drawbacks of the first generation photosensitizer Photofrin, chlorophyll-a derivatives become the promising compounds in the development of novel photosensitizing drugs for PDT [13–15]. Chlorophyll-a derivatives have several advantages such as light relatively deep penetration within tissues via absorption of light of longer wavelength, selective accumulation in target tissue and its minimal side effect [16,17].

Herein, we report the antitumor activity of a chlorophyll-a derivative, 3-[1-hydroxyethyl]-3-devinylpyropheophorbide-a (HEPa) (Fig. 1). Photophysical characteristics of HEPa were

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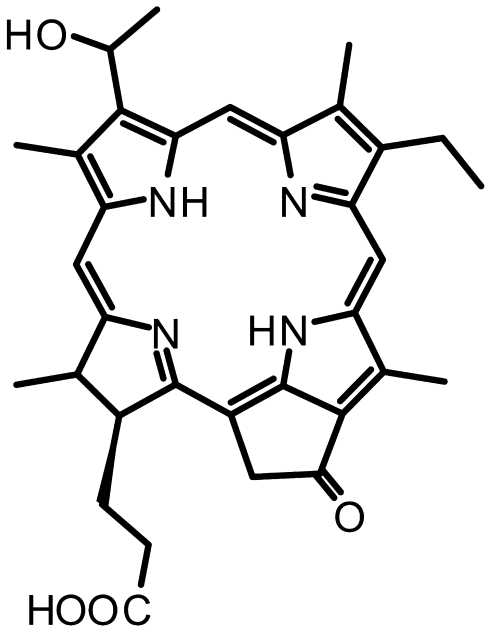

Fig. 1. Chemical structure of HEPa.

measured. And its cytotoxicity, intracellular localization, biodistribution, efficiency of photodynamic therapy (PDT), histological analysis were investigated using human bile duct carcinoma cells (QBC-939) and QBC-939 tumor bearing BABL/c nude mice as animal model.

2. Materials and methods

All the chemicals and reagents were of analytical grade and used without any purification, and all the reagents were obtained from Sinopharm Chemical Reagent Co., Ltd.

^{1}H NMR spectra were measured at 400 MHz on an Advance II spectrometer. The chemical shifts are reported in ppm (δ) and coupling constant (J) values are given in hertz (Hz). Mass spectra (MS) were recorded on a Shimadzu LCMS-2010EV single quadrupole benchtop mass spectrometer using positive electrospray ionization.

2.1. Chemistry

2.1.1. Procedure for the synthesis of compound 3-[1-hydroxyethyl]-3-devinylpyropheophorbide-a (HEPa)

125 mg pyropheophorbide-a (0.23 mmol) were added into 10 mL 30% HBr/acetic acid solution, and stirred at 40 °C for 5 h. The acid and solvent were removed under reduced pressure. The resulting residue was stirred with 20 mL distilled water at room temperature for 0.5 h. Methyl alcohol (20 mL) and 2 M LiOH (5 mL) were added. The solution was refluxed for 2 h in a water bath under nitrogen atmosphere. Acetic acid was added to adjust the pH to 6–7. The mixture was added 30 mL dichloromethane, washed with saturated salt water (60 mL × 3), dried with anhydrous sodium sulfate and filtered. The organic solvent of the filtrate was evaporated in vacuum. The residue was chromatographed using CH_2Cl_2/MeOH (30:1, v/v) as eluents in 76% (84 mg) yield as dark brown powder. ESI–MS (*m*/*z*): 552.1981[M+1]$^+$. ^{1}H NMR (δ, DMSO, ppm): 12.13 (s, 1H, −COOH), 9.85 (d, *J* = 14.0 Hz, 1H, *meso*-H), 9.75 (s, 1H, *meso*-H), 8.79 (s, 1H, *meso*-H), 6.32 (s, 1H, −OH), 6.11 (q, 1H, 3^a-H), 5.25-5.09 (m, 2H, 13^2-H), 4.59-4.57 (m, 1H, 18-H), 4.32-4.31 (m, 1H, 17-H), 3.74 (q, *J* = 7.5 Hz, 2H, 8^a-H), 3.63 (s, 3H, 12-CH_3), 3.40 (s, 3H, 2- CH_3), 3.23 (s, 3H, 7-CH_3), 2.67-2.60 (m, 2H, 17^b-H), 2.36-2.29 (m, 2H, 17^a-H), 2.00 (d, *J* = 3.8 Hz, 3H, 3^b- CH_3), 1.79–1.73 (d, 3H, 18- CH_3), 1.65 (t, *J* = 7.5 Hz, 3H, 8^b- CH_3), −1.95 (s, 2H, NH)

2.2. UV–vis absorption and fluorescence spectra

UV–vis absorption spectrum was recorded on an ultraviolet visible spectrophotometer (Model V-530, Japan). Fluorescence spectra were measured on a Fluorescence Spectrometer (FluoroMax-4, France). Slits were kept narrow to 1 nm in excitation and 1 or 2 nm in emission. Right angle detection was used. All the measurements were carried out at room temperature in quartz cuvettes with path length of 1 cm. HEPa was dissolved in *N*,*N*-dimethylformamide (DMF) to get 6 μM solutions.

2.3. Singlet oxygen quantum yield

1,3-Diphenylisobenzofuran (DPBF) was used as a $^1O_2$ trapping reagent in DMF solution. In a typical experiment, 2 mL DMF solution containing 20 μM DPBF and 0.5 μM HEPa was placed in a sealed quartz cuvette. A 5 mW Nd:YAG laser (650 nm) was used as the light source. The absorbance of the solution at 410 nm was measured every 10 s for a 120 s period with an ultraviolet visible spectrophotometer [18]. The decrease of the absorbance caused by photobleaching of DPBF was measured and corrected in all experiments. The natural logarithm values of absorption of DPBF at 410 nm were plotted against the irradiation time and fit by a first-order linear least-squares model to get the singlet oxygen generation rate of the photosensitized process [19]. The $^1O_2$ quantum yield of HEPa in DMF was calculated using Methylene blue as a standard.

2.4. Biological study

2.4.1. Cell line and culture conditions

Human cholangiocarcinoma cell line QBC-939 was obtained from the Type Culture Collection of the Chinese Academy of Sciences. All cell culture related reagents were purchased from Shanghai Ming Rong Bio-Science Technology Co., Ltd. Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic (penicillin-streptomycin) in a CO_2 incubator with 5% CO_2 at 37 °C.

2.4.2. In vitro phototoxicity assay

QBC-939 cells (2×10^4 cells/well) were seeded in 96-well plates in 200 μL DMEM medium, and allowed to attach for 24 h. DMEM medium containing HEPa in different concentrations (0, 0.5, 1, 2, 4, 8 μM) was administered to cells and allowed to uptake for 24 h. DMEM medium containing HEPa was removed and cells were washed with PBS before irradiation with different light doses (0, 2, 4, 8 J/cm^2) using an Nd: YAG laser at 650 nm. Then, the cells were further incubation for 24 h. The resulting dark or photoxic effects on cells were evaluated via 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-terazolium bromide (MTT) colorimetric assay. Untreated cells served as 100% viable cells. Data presented are averaged results of triplicate experiments.

2.4.3. Intracellular localization

QBC-939 cells grown on coverslips were incubated with medium containing HEPa in 5 μM for 4 h at 37 °C in dark, then rinsed in the medium and incubated with 1 μM Lyso Tracker Blue (Beyotime, Nanjing, China) for 20 min and 1 μg/ml Hoechst 33342 (Beyotime, Nanjing, China) for 10 min at 37 °C, respectively. After washing with PBS, coverslips were fixed for 10 min at 4 °C with 4%