Journal of Photochemistry and Photobiology B: Biology 151 (2015) 186–193

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Effects of a novel porphyrin-based photosensitizer on sensitive and multidrug-resistant human gastric cancer cell lines

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ARTICLE INFO

Article history:
Received 3 April 2015
Received in revised form 13 August 2015
Accepted 14 August 2015
Available online 19 August 2015

Keywords:
Photodynamic therapy
DTP
Multidrug resistance
Cytotoxicity

ABSTRACT

Photodynamic therapy (PDT) has been considered to be a possible candidate approach in combating multidrug resistance (MDR) phenomenon during the treatment of cancer. To investigate the photocytotoxicity of a novel porphyrin-based photosensitizer, meso-5-[ρ-DTPA-aminophenyl]-10, 15, 20-triphenyl-porhyrin (DTP) (Fig. 1A), on MDR cells, the intracellular DTP uptake, phototoxicity and subcellular DTP localization were studied by using a human gastric cancer MGC803 cell line and its paclitaxel selected subline MGC803/PA expressing MDR phenotype. No significant difference was observed in intracellular DTP accumulation between sensitive and resistant cell lines after exposure to 1.56 μM concentration for 6 h. DTP-PDT induced significant photocytotoxicity on both MGC803 and MGC803/PA cell lines and the photokilling was greater in MGC803 cell line in comparison to MGC803/PA. The fluence that caused 50% cell death was 4.42 and 6.29 J/cm^2 in MGC803 and MGC803/PA cell lines, respectively. The presence of Pgp inhibitors verapamil and cyclosporin A could not modify the intracellular DTP level in MGC803/PA cell line and the phototoxic effects. DTP was localized at lysosomes of MGC803 cell line but at lysosomes and mitochondria of MGC803/PA. Our results indicated that DTP-mediated PDT could eradicate gastric cancer cells whether or not they express MDR although the efficacy is slightly reduced in the MDR cells. The photokilling in MDR cells could not be altered by MDR inhibitor verapamil. The slightly different photocytotoxicity between sensitive and resistant cell lines could not explained by classical Pgp MDR and might be attributed to the differential intracellular DTP localization sites.

1. Introduction

Photodynamic therapy (PDT) is a promising modality for the treatment of malignant tumors. It typically involves the combination of a non-toxic photosensitizing agent and a harmless visible light, which, in the presence of oxygen, results in the generation of singlet oxygen and other reactive oxygen species (ROS) [1]. These ROS are responsible for PDT-based cell killing which eventually leads to tumor ablation [2].

Multidrug resistance (MDR) is a huge obstacle encountered during cancer chemotherapy for some types of chemotherapeutic agents used clinically. MDR frequently refers to elevated expression of a transmembrane P-glycoprotein (Pgp), which could pump its substrate drugs out of cells and consequently resulted in intracellular concentrations decreased and chemotherapy failure [3,4]. A wide range of antitumor drugs, with structural and functional difference, are substrates for Pgp [5,6]. Currently, MDR has been one of the main reasons limiting the efficacy and application of some chemotherapeutic drugs during cancer treatment. A new method, which could overcome this multidrug resistance, is in an urgent need.

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http://dx.doi.org/10.1016/j.jphotobiol.2015.08.020
1011-1344/

PDT has been considered to be an alternative for treatment of resistant cancer since its mechanism is completely different from that of conventional chemotherapy. In fact, some studies have showed that PDT is effective in treating MDR cancer cells with mesoporphyrin, phthalocyanines and Photofrin [7,8]. Different levels of photocytotoxicity were obtained for these photosensitizers in resistant cell lines in comparision to their parental cell lines [9–11]. Meanwhile, cross-resistance to chemotherapeutic drugs and several photosensitizers, in particular porphyrin derivatives, in chemo-resistant cells with elevated Pgp expression, has been also reported [11,12]. The failure of PDT in MDR cells could be attributed to an impaired accumulation of sensitizers caused by their exclusion by Pgp efflux protein, or to other mechanisms that are not directly related to over-expressed Pgp [10,13].

Photosensitizer is an important component for PDT. Recently, we have developed a novel photosensitizer named meso-5-[ρ-DT PA-aminophenyl]-10,15,20-triphenyl-porhyrin (DTP). This compound was a porphyrin derivative. Our previous studies demonstrated DTP-induced cytotoxic effects on several human sensitive gastric cancer cell lines [14], however, whether it has therapeutical effects on drug resistant human gastric cancer cell lines is still not investigated. Our present work inspects the efficacy of DTP-based photosensitization to combat multidrug resistance. This objective was achieved by comparing intracellular DTP accumulation, photocytotoxicity and subcellular localization in human gastric cancer MGC803 cell line and its paclitaxel resistant subline, MGC803/PA, which is characterized by Pgp over-expression.

2. Materials and methods

2.1. Synthesis of DTP

The synthetic process of DTP was obtained in detail according to our patent specifications (CN 201010135433.X) and its chemical structure was presented in Fig. 1A. Firstly, we synthesized meso-5,10,15,20-tetraphenyl-porhyrin in propionic acid solvent by classical Adler method. Meso-5,10,15,20-tetraphenyl-porhyrin (1 equiv) was added and stirred in 20 ml dichloromethane under −10 °C until dissolution, and 65% concentrated nitric acid (1 equiv) was dropped into the solution. The nitrated product was reduced with sodium nitrite in dichloromethane at room temperature and then a reduced mixture was gained. The meso-5-(ρ-aminophenyl)-10,15,20-triphenyl-porhyrin was obtained as the main product after separating the mixture by silica gel chromatography. 0.2 g meso-5-(ρ-aminophenyl)-10,15,20-triphenyl-porhyrin, 0.446 g diethylenetriaminepentaacetic acid dianhydride (DTPAA), 0.046 g dimethylaminopyridine (DMAP) and 0.040 g triethylamine (TEA) were dissolved in 10 ml dimethyl sulfoxide (DMSO) in 25 ml flask together. After stirred over 12 h at room temperature, 5 ml H_2O was added into the reaction solution and the precipitate was formed. The final product DTP was obtained by crystallizing the precipitate in the mixed solution of DMSO and water. The chemical structure of DTP was shown in Fig. 1 and its molecular weight was 1004.39. DTP was dissolved with RPMI-1640 medium and stored at 12.5×10^3 μM at 4 °C protected from visible light. All the chemicals and reagents used during synthesis of DTP were obtained from Sigma–Aldrich. They were of analytical grade and used without any purification.

2.2. Spectral analysis of DTP

DTP solution was prepared in RPMI 1640 medium or the mixture of DMSO and water (1:1) to get a 3.12 μM concentration and 100 μl was added into a 96 well plate. UV–Vis absorption spectrum was recorded on an ultraviolet visible spectrophotometer (Thermo NanoDrop 2000, USA).

2.3. Cell preparation and culture conditions

Human gastric cancer MGC803 cell line was obtained from the Chinese Academy of Sciences and its paclitaxel resistant subline, MGC803/PA, was derived by continuous exposure to paclitaxel (PA) (Gibco, USA) with increasing concentrations from 0.0125 to 0.5 μg/ml for total 4 months [15]. The multidrug-resistant MGC803/PA cell line was identified as P-glycoprotein over-expression by antibody staining on flow cytometry, showing 98.7% positive (data shown in results). To further confirm the successful preparation of paclitaxel resistance, rhodamine 123 efflux test was performed (shown in results). Both cell lines were grown in RPMI 1640 medium (Solarbio, China) supplemented with 10% fetal calf serum (FCS) (Gibco, USA) and maintained at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. The resistant subline, MGC803/PA, was cultured in 0.5 μg/ml paclitaxel-containing medium, trypsinized and re-seeded into fresh medium every 3 days. Before used in formal experiments, the cells underwent a wash-out period of 10 days in paclitaxel-free medium to allow sufficient paclitaxel efflux from cells.

2.4. Confirmation of paclitaxel-resistant cell line

2.4.1. Rhodamine 123 efflux test

MGC803 and MGC803/PA cell lines, seeded into 6-well plates, were incubated with 5 mM rhodamine 123 (Solarbio, China) for 30 min at room temperature in the dark. The cells were washed with PBS (Solarbio, China) and then visualized to detect the difference in fluorescence within sensitive and resistant cell lines under an DMIRB inverted fluorescence microscopy (Leica, Germany) equipped with a 50 W mercury vapor lamp. Rhodamine 123 emitted red fluorescence after laser irradiation and its excitation and

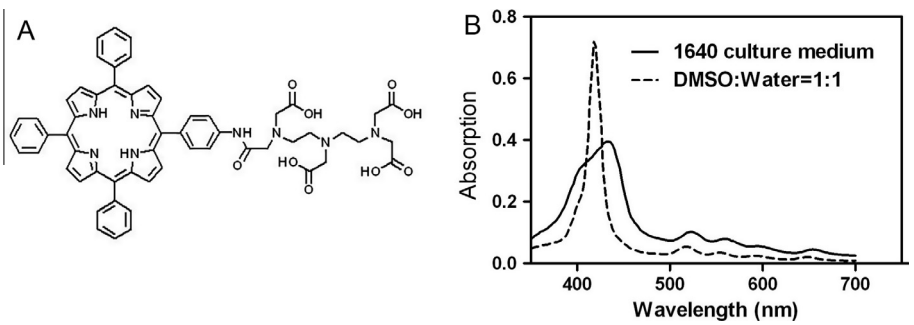

Fig. 1. Basic information of photosensitizing agent DTP. A: Chemical structure; B: UV–Vis absorption spectrum in RPMI 1640 medium or the mixture of DMSO and water (1:1) at 3.12 μM.