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A hematoporphyrin-based delivery system for drug resistance reversal and tumor ablation

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ABSTRACT

Nanotechnology-based drug delivery systems have been intensively investigated, while only a few of them can be used for clinic application. Hematoporphyrin (HP), a major molecule in erythrocyte, has been widely used in photodynamic therapy (PDT). In the present study, polyethylene glycol (PEG) modified hematoporphyrin (HPP)-based nanoparticle system was designed to load doxorubicin (HPPD), in achieving a synergistic effect of chemotherapy and PDT. Herein we presented that HPPD formed narrowly dispersed nanoparticles at 35 ± 2 nm, yielding an enhanced drug release at pH5.8 along with laser radiation. This combined treatment with HPPD and radiation facilitated drug penetration to the nucleus thereby reducing 12-fold decrease in IC_{50} value and promoting apoptosis in drug-resistant breast cancer cells. Notably, little toxicity was detected with HPP at the cellular level and in animal models. Live animal imaging revealed that HPPD performed ultra high tumor uptake in both mice and marmoset models. Strikingly, intravenous administration of HPPD and radiation on the tumor achieved efficient tumor ablation, without inducing myocardial injury. We report here the development of a biomolecule, HP-based nanoparticle system, which can synergistically yield chemotherapy and PDT.

1. Introduction

Chemotherapy remains as a major treatment modality for cancer, yet its efficacy is limited by the associated toxic side effects as well as the evolution of drug-resistant tumors after prolonged treatments [1,2]. Also, chemotherapy is ineffective against certain tumor classes such as hepatoma, due to chemotherapy-deactivating enzymes liver cells. Developing targeted drug delivery system (DDS) therefore becomes a key strategy to reduce the toxicity [3–5]. Prolonging the drug retention time inside the cancer cells can help overcome drug resistance and enhance killing efficacy against hepatoma [6–8]. Recently, numerous nanoparticle-based DDSs have been developed to extend the blood circulation half-time, lower toxicity and increase tumor targeting ability [9,10]. Nevertheless, few have resulted in significant improvements in patient survival rates [11]. Several photo-thermal systems, such as graphene oxide, FeCo/graphite, and Au nanoparticles (NPs), were also designed as drug carriers to achieve a combined chemo/photothermal therapy [12–15]. Yet, the biosafety of these nanoparticles remains to be addressed prior to their possible clinical applications.

Photodynamic therapy (PDT) has been attempted in clinical practice to combat cancers and showed limited efficacy in reversing drug resistance [16,17]. Hematoporphyrin (HP), a major biomolecule in erythrocytes, is enriched in tumor regions and can kill cancer cells by generating reactive oxygen species [18]. In the current work, PEGylated HP (HPP) was adapted as a tumor-targeting drug delivery platform and verified in various tumor models. Here we demonstrated that the self-assembled nanoparticle could

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efficiently improve drug accumulation, reverse drug resistance, display high level of tumor uptake and efficient tumor ablation in combination with low-power laser radiation without inducing myocardial injury, which may be translated into clinic to combat tumors.

2. Materials and methods

2.1. Materials and cell lines

Hematoporphyrin was purchased from Frontier Company. Bis (3-aminopropyl) terminated PEG solution (Mw: 2000) was supplied by Sigma–Aldrich. Both the doxorubicin (Dox) sensitive breast cancer cell line MCF-7 and its dox-resistant cell line ADR/MCF-7 were kindly gifts from the Detroit Hospital (Detroit, MI, USA). ADR/MCF-7 cells were maintained in RPMI 1640 medium (RPMI-L-Glutamine, Invitrogen) supplemented with 10% v/v fetal bovine serum (FBS, Sigma–Aldrich), 1% v/v penicillin/streptomycin (Sigma–Aldrich), 0.8ug/mL doxorubicin (Dox, Sigma–Aldrich). MCF-7 human breast cancer cells and MHCC-97H human hepatoma cancer cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM-high glucose, Invitrogen) with high glucose, 10 v/v% FBS, 1 v/v% penicillin/streptomycin.

2.2. Synthesis and characterization of HPP

HPP nanoparticles were formed upon addition of an aqueous HP solution (1.0 mg/mL, dissolved in 0.2 M morpholino ethane sulfonic acid solution) to NH_2 terminated PEG solution (4.0 mg/mL, dissolved in 0.2 M phosphate solution) at a volume ratio of 1: 4, under stirring over night. Excess PEG was removed by centrifuge filtration through Amicon centrifugal filters (Millipore) with 3 kDa molecular weight cut off (MWCO) and washed with deionized water (DDW) for 6 times.

2.3. Synthesis of drug loaded HPP (HPPD)

Dox was dissolved in DDW at 1 mg/mL and then mixed with HPP at volume ration of 1: 2. Free drug was removed by centrifuge filtration (Millipore, 3 kDa). The final concentration of doxorubicin in HPP was 0.25 mg/mL as determined by high-performance liquid chromatography (HPLC).

2.4. Evaluation of the drug release profiles

To determine the kinetics of doxorubicin release from HPP, 2 mL HPPD was placed in a dialysis bag (molecular mass cut off 1000 Da) and incubated in 150 mL of PBS buffer (pH 7.4 or pH 5.8) at 37 °C at the speed of 100 ± 5 rpm, and aliquots of incubation media were removed at predetermined time points. The content of drug released was fluorimetrically determined using reverse-phase HPLC with a C18 column, with acetic acid, methanol, and 10 mM Na_2HPO_4 (13:19:68, vol/vol/vol, pH 4.0) as the eluent solution. And the detection wavelength was set at 480 nm.

2.5. In vitro cytotoxicity evaluation

The light source (irradiation) for activating the HPPD was obtained from Tianjin Biotech (FC-63003, High Power Devices, China), emitting in the spectral region of 600–660 nm, with the highest intensity at about 633 nm. The irradiation rate was set at 100 mW/cm^2. The diameter of the spots could be monitored, which made it easy to adjust different area of cell culture plates. In order to make sure the uniformity of all samples illuminated, the vertical distance between the cultures plates and light source was fixed at 5 cm, thereby all the cells could get same irradiation level.

For cell viability study, ADR/MCF-7 cells were seeded in 96-well plate at a density of 6.0 × 10^3 cells per well and cultured for 24 h. Then culture medium was

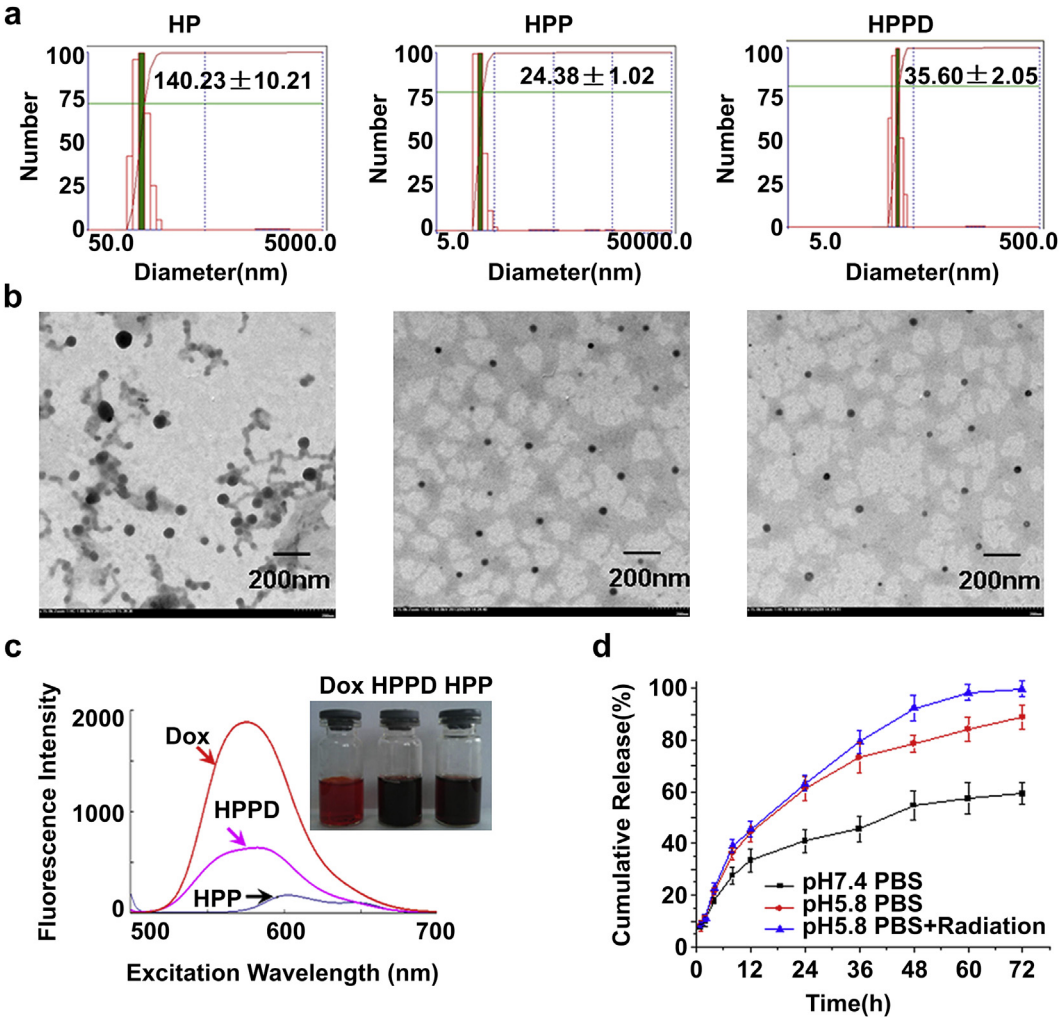

Fig. 1. HPPD NPs for tumor targeting. (a) Particle sizes of HP, HPP and HPPD measured by DLS. (b) TEM images of HP, HPP and HPPD of sphere shape. (c) UV–VIS–NIR spectra of Dox, HPP and HPPD solution. HPPD has high optical emission absorption around 580 nm. Inset: a photo of Dox, HPP and HPPD solution at the concentration of 0.5 mg/mL (d) Dox release profile of HPPD at pH7.4, and 5.8 in the absence or presence of laser radiation in PBS at 37°C.