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Reversal of doxorubicin resistance in breast cancer by mitochondria-targeted pH-responsive micelles

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ABSTRACT

Chemotherapy is an important approach for clinical cancer treatment. However, the success of chemotherapy is usually hindered by the occurrence of intrinsic or acquired multidrug resistance of cancer cells. Herein, we reported an effective approach to overcome doxorubicin (DOX) resistance in MCF-7/ADR breast cancer using DOX-loaded pH-responsive micelles. The micelles were prepared from a pH-responsive diblock copolymer, poly(ethylene glycol)-block-poly(2-(diisopropylamino)ethyl methacrylate) (PEG-*b*-PDPA), and a vitamin E derivate (D-α-tocopheryl polyethylene glycol 1000 succinate, TPGS) (denoted as PDPA/TPGS micelles). At neutral pH of 7.4, DOX was loaded into the hydrophobic core of PDPA/TPGS micelles via a film sonication method. After cellular uptake, the DOX payload was released in early endosomes by acidic pH-triggered micelle dissociation. Meanwhile, the TPGS component synergistically improved the cytotoxicity of DOX by targeting mitochondrial organelles and reducing the mitochondrial transmembrane potential. In vitro cell culture experiments using DOX-resistant MCF-7/ADR cells demonstrated that PDPA/TPGS micelles reduced the IC_{50} of DOX by a sixfold magnitude. In vivo animal studies showed that DOX-loaded PDPA/TPGS micelles (PDPA/TPGS@DOX) inhibited tumor growth more efficiently than free DOX in a nude mouse model bearing orthotopic MCF-7/ADR tumor. All these results imply that the mitochondria-targeted pH-responsive PDPA/TPGS micelles have significant potential for efficiently combating DOX resistance in breast cancer cells.

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1. Introduction

The chemotherapy is one important approach for clinical cancer treatment by inhibiting the growth of primary tumor and suppressing the proliferation of metastatic tumor cells [1,2]. Although a large number of anticancer drugs have been clinically approved so far, doxorubicin (DOX) is being actively investigated owing to its wide anticancer spectrum and superior cytotoxicity to various cancer cells [3–5]. Unfortunately, the therapeutic effect of DOX is usually neutralized by its severe cardiotoxicity and the occurrence of intrinsic or acquired drug-resistance in cancer cells [6,7]. The enhanced removal of DOX from cancer cells is identified as a major mechanism of DOX resistance, and is generally attributed to the overactivation of a superfamily of energy-dependent ATP-binding cassette transporters, such as P-glycoprotein (P-gp) [8]. To address this challenge, several P-gp inhibitors had been used to reverse DOX resistance. However, only moderate effects have been achieved, partly due to the low tumor specificity and high inherent toxicity of the P-gp inhibitors [9–12].

Mitochondrion, a critical subcellular organelle, is usually referred to the cellular power source since it controls ATP synthesis [13]. The evidence accumulated to date indicates that mitochondria-associated apoptosis and energy metabolism could play important roles in cancer chemotherapy since the mitochondrion is also a crucial regulator of cell death [14–17]. Furthermore, recent studies conducted by several groups, including ours, have demonstrated that the mitochondria could be an alternative target to surmount multidrug resistance by suppressing the mitochondria's function of producing ATP and subsequently inhibiting the drug efflux function of P-gp protein [18,19]. For example, several vitamin E derivatives have been found to enhance the therapeutic effect of anticancer drugs by reducing the transmembrane potential of mitochondria and thereby suppressing their ATP-producing activity [20–22]. Owing to this remarkable property of the vitamin

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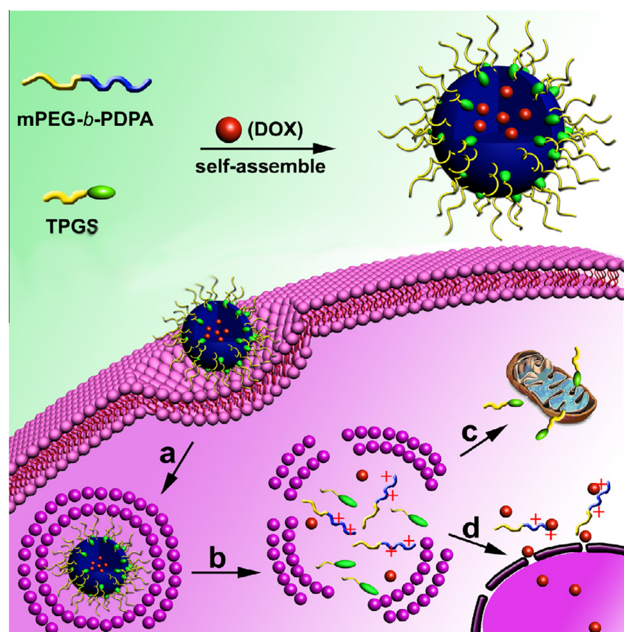
E derivatives, they have been used as functional excipients for the controlled delivery of several kinds of anticancer drugs [20,23–25]. However, the co-administration of vitamin E derivatives with anticancer drugs to reverse drug resistance has rarely been exploited.

Recently, we developed a set of pH-responsive micelles containing a poly(2-(diisopropylamino)ethyl methacrylate) (PDPA) segment for the efficient delivery of photosensitizer or small interfering RNA (siRNA) by targeting the tumor or the intracellular acidic microenvironment [26,27]. All these micelles were specifically dissociated in early endosomes (pH 5.9–6.2) for timely payload release by protonation of the diisopropyl-substituted tertiary amino groups [28,29]. More interestingly, one such micelle, prepared from poly(2-(dimethylamino)ethyl methacrylate)-block-poly (2-(diisopropylamino)ethyl methacrylate) (PDMA-*b*-PDPA) diblock copolymers, had been successfully applied for the co-delivery of paclitaxel and siRNA-Bcl-2 to reverse paclitaxel resistance in A549 lung cancer cells [30]. In the current study, pH-responsive micelles with mitochondria-targeting ability were developed for DOX delivery in an attempt to combat DOX resistance in MCF-7/ADR breast cancer cells. The micelles were prepared from polyethylene glycol-block-poly(2-(diisopropylamino)ethyl methacrylate) (PEG-*b*-PDPA) diblock copolymer and a vitamin E derivate (D-α-tocopheryl polyethylene glycol 1000 succinate, TPGS). As illustrated in Scheme 1, upon cellular uptake, PDPA/TPGS micelles released DOX in early endosomes by acidic pH-induced dissociation of PEG-*b*-PDPA molecules. Meanwhile, the TPGS moiety in the PDPA/TPGS micelles reduced the mitochondrial transmembrane potential by targeting mitochondrial organelles, thereby synergistically sensitizing the MCF-7/ADR breast cancer cells to DOX treatment. The pH-responsive property of the hybrid micelles was thoroughly characterized by particle size measurement, morphology characterization and fluorescence examination, respectively. The ability of the pH-responsive micelles to reverse DOX resistance in breast cancer cells was tested in vitro and in vivo by using a MCF-7/ADR breast cancer cell line and a nude mouse model bearing an orthotopic MCF-7/ADR tumor.



Scheme 1. The preparation and mechanism of mitochondria-targeted pH-responsive PDPA/TPGS@DOX micelles to overcome DOX resistance in breast cancer cells. (a) Cellular uptake; (b) protonation-triggered dissociation of PDPA/TPGS@DOX micelles inside endosome/lysosome vesicles; (c) mitochondria targeting of TPGS; and (d) nuclear entry of DOX molecules.

2. Materials and methods

2.1. Materials

Methoxy polyethylene glycol 5000 ($mPEG_{113}$-OH), copper bromide (CuBr, 99.99%), DNA-free RNase A, propidium iodide (PI), sulforhodamine B (SRB), Nile red (NR), TPGS and pentamethyl diethylenetriamine were all purchased from Sigma–Aldrich (Shanghai, China) and used as received. Dialysis tubing (molecular weight cutoff (MWCO) = 3.5 Da) and ultracentrifugation units with an MWCO of 30 kDa were ordered from Fisher Scientific. Inc. (IL, USA). Hoechst 33342 was ordered from Life Technologies (Shanghai, China). RPMI 1640 medium, 0.25% Trypsin–0.02% EDTA, fetal bovine serum (FBS) and an Alexa Fluor® 488 Anexin V/PI Dead Cell Apoptosis Kit were all obtained from Gibco (Invitrogen, USA). 2-Diisopropylaminoethyl methacrylate was purchased from Scientific Polymer Products Inc. (NY, USA). All other chemicals and regents were analytical grade and obtained from Sinopharm Group Chemical Reagent Co., Ltd (Shanghai, China).

2.2. Cell lines and cell culture

The parental MCF-7 human breast cancer cell line was obtained from the cell bank of the Chinese Academy of Sciences (Shanghai, China). The DOX-resistant MCF-7 cell line (MCF7/ADR) was purchased from Keygen Biotech Co. Ltd (Shanghai, China). MCF-7 cells were cultured in complete RPMI 1640 cell culture medium containing 10% FBS, 100 U ml^{-1} penicillin G sodium and 100 μg ml^{-1} streptomycin sulfate. MCF-7/ADR cells were maintained in the same culture medium as used for MCF-7 cells but with the addition of 1.0 μg ml^{-1} DOX. Both kinds of cells were maintained in a 37 °C incubator under a humidified atmosphere with a 5% CO_2 supply. All experiments were performed in the logarithmic phase of cell growth.

2.3. Preparation and characteristics of PDPA/TPGS micelles

$mPEG_{113}$-*b*-$PDPA_{10}$ diblock copolymer was synthesized by an atom transfer radical polymerization (ATRP) method, as described previously [26]. To optimize the chemical composition of the PDPA/TPGS micelles, $mPEG_{113}$-*b*-$PDPA_{10}$ diblock copolymer and TPGS were mixed at various weight ratios and co-dissolved in chloroform overnight. The chloroform solution was vacuum dried to form a thin film, which was then hydrated with deionized water for 10 min and vortexed for 30 s to obtain PDPA/TPGS micelles. The scattering intensity, hydrodynamic size distribution and zeta potential of the micelles were examined at different pHs using dynamic light scattering (DLS; Nanosizer, Malvern, UK). To investigate acidic pH-triggered dissociation of PDPA/TPGS micelles, 1.0 wt.% NR was loaded into PDPA/TPGS micelles following the same procedure as described above. The fluorescence images of the NR-loaded micelles at different pHs were photographed using a UVP bioimage system (Upland, CA). The fluorescence emission of the NR-loaded PDPA/TPGS micelles was examined using fluorescence spectrophotometry (excitation at 475 nm, emission at 500–580 nm; Hitachi F-4600, Japan).

To prepare DOX-loaded PDPA/TPGS micelles (denoted as PDPA/TPGS@DOX), PEG_{113}-*b*-$PDPA_{10}$ copolymer and TPGS were mixed at a weight ratio of 80/20 and co-dissolved in chloroform overnight. The polymer solution in chloroform was mixed with DOX solution in methanol to form a homogeneous solution. The mixed solution was vacuum dried, hydrated with deionized water for 10 min and vortexed for 30 s to form PDPA/TPGS@DOX. DOX-loaded PDPA micelles without TPGS (PDPA@DOX) were also prepared as the control. Any unencapsulated DOX was removed by ultrafiltration,

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