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TPGS functionalized mesoporous silica nanoparticles for anticancer drug delivery to overcome multidrug resistance

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ABSTRACT

Multidrug resistance (MDR) has become a very serious problem in cancer therapy. To effectively reverse MDR in tumor treatments, a new pH-sensitive nano drug delivery system (NDDS) composed of mesoporous silica nanoparticles (MSNs) and D-a-tocopheryl poly-ethylene glycol 1000 succinate (TPGS) copolymers was synthesized to deliver doxorubicin (DOX) into drug-resistant breast cancer cell line (MCF-7/ADR). DOX@MSNs-TPGS were characterized to have a single peak size distribution, high DOX loading efficiency and a pH-dependent drug release profile. MSNs-TPGS were internalized via caveolae, clathrin-mediated endocytosis and energy-dependent cellular uptake. The DOX@MSNs-TPGS exhibited 10-fold enhanced cell killing potency compared to free DOX and DOX@MSNs. The enhanced MDR reversal effect was ascribed to the higher amount of cellular uptake of DOX@MSNs-TPGS in MCF-7/ADR cells than that of free DOX and DOX@MSNs, as a result of the inhibition of P-gp mediated drug efflux by TPGS. In vivo studies of NDDS in tumor-bearing mice showed that DOX@MSNs-TPGS displayed better efficacy against MDR tumors in mice and reached the tumor site more effectively than DOX and DOX@MSNs, with minimal toxicity. These results suggest DOX@MSNs-TPGS developed in this study have promising applications to overcome drug resistance in tumor treatments.

1. Introduction

Breast cancer is the most common cancer and the primary cause of cancer death among females in the world, with an estimated 1.7 million cases and 521,900 deaths in 2012. Breast cancer accounts for 25% of all cancer cases and 15% of cancer deaths among females [1,2]. Up to now, chemotherapy remains one of the most effective treatments for breast cancer.

However, traditional anti-cancer drugs for combating the tumor usually lead to negative therapeutic effect by three obstacles: lack of selectivity to cancer cells and normal cells, which would induce severe side effects towards normal tissues and organs [3,4]; the second limitation is that these anti-tumor agents are rapidly removed from the blood; the last but the largest trouble is the multi-drug resistance (MDR) commonly caused by tumor cells, thus leading to the reduced drug accumulation and therapeutic effect [5,6].

Overexpression of P-glycoprotein (P-gp) is the main mechanism of MDR in cancers [7]. P-gp is encoded by the MDR1 gene whose product is identified as an energy-transducing transport pump. P-gp can promote the efflux of anti-tumor drugs in an ATP-dependent manner. Therefore, it is very necessary to overcome P-gp-mediated MDR and maintain the accumulation of chemotherapeutic drugs in cancer cells [8].

In order to overcome MDR over the past decades, considerable attempts have been made to develop P-gp inhibitors [9,10]. Although many inhibitors can overcome drug resistance in vitro, the results in vivo have been disappointing, mainly because of the poor aqueous solubility of inhibitors and low intracellular concentration of inhibitors at targeted sites [2,11,12]. More importantly, MDR inhibitors may cause undesired side effects to normal cells. Recently, several nonionic surfactants have been demonstrated to influence the pharmacokinetics of drugs by inhibiting P-gp efflux pumps to regulate cellular drug transport activity in the order TPGS > Pluronic PE8100 > Cremophor EL > Pluronic PE6100 approximately Tween 80 [13,14]. TPGS (D-a-tocopheryl poly-ethylene glycol 1000 succinate), a water-soluble derivative of natural vitamin E has become to be one of the most effective

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commercially available P-gp inhibitor surfactants [15,16]. It is formed by esterification of vitamin E succinate with polyethylene glycol (PEG) 1000. The co-administration of TPGS could inhibit P-gp mediated MDR [16,17].

As it is well known, targeted drug delivery systems using nanocarriers in the anti-tumor treatment have achieved great success in recent years [18–24]. Nano drug delivery systems (NDDS) tend to accumulate in tumors because of the enhanced permeability and retention (EPR) effect, thereby increasing the targeting ability of the loaded drugs [25,26]. In addition, the modification with PEG on the surface of NDDS would give them longer circulating time in vivo and improve the distribution of anti-cancer drugs in tumor tissues [27]. To some extent, NDDS are also expected to avoid MDR by bypassing P-gp [28–30]. However, free drugs released from the nanocarriers into the cytoplasm are re-exposed to the efflux pump, partially reducing therapeutic effect [31].

Among the reported NDDS, mesoporous silica nanoparticles (MSNs) have been shown to be superior hosts for drug molecules because of their distinctive mesoporous structure, large surface area, tunable pore size, well-defined surface properties and good biocompatibility in vivo [32,33]. However, in our preliminary results, we found that MSNs increased the uptake of doxorubicin (DOX) in MCF-7 cells (DOX sensitive) only, while drug accumulation in MCF-7/ADR (DOX resistance) cells still remained very low. Therefore, we need to develop a novel and multifunctional drug delivery platform that may be suitable to overcome MDR and enhance its efficacy to breast cancer treatment.

Based on above considerations, we designed TPGS-functionalized MSNs for loading DOX to overcome the MDR of tumors. DOX was investigated as a model anticancer drug. DOX@MSNs-TPGS were characterized using dynamic light scattering (DLS), transmission electron microscopy (TEM) and UV/VIS spectroscopy. The in vitro release behaviors of DOX@MSNs-TPGS were measured at PBS (pH 7.4 and 5.0). The cellular uptake and internalization mechanisms of MSNs-TPGS were also investigated. In addition, we studied the in vivo biodistribution and tumor targeting activities of Cy5.5-labeled MSNs-TPGS and evaluated pharmacokinetic (PK) profiles of DOX@MSNs-TPGS compared to those of free DOX. Finally, anti-tumor efficacies of DOX@MSNs-TPGS were also assessed in vivo.

2. Materials and methods

2.1. Materials

Tetraethylorthosilicate (TEOS), cetyltrimethylammonium bromide (CTAB), sodium chloride (NaCl), methanol, sodium hydroxide (NaOH), dichloromethane (DCM), acetonitrile, dimethyl sulfoxide (DMSO), D-α-tocopheryl poly-ethylene glycol 1000 succinate (TPGS), phosphate buffered saline (PBS, pH 7.4; pH 5.0), Triton X-100, trypsin–EDTA solution, MTT and Cy5.5 were purchased from Sigma-Aldrich. Doxorubicin (DOX) was obtained from Sike Pharmaceutical Co., Ltd. (Jiangsu, China). All cell culture medium were obtained from Gibco/Invitrogen (Grand Island, NY, USA).

Human breast cancer DOX-sensitive MCF-7 cells and DOX-resistant MCF-7/ADR cells were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). MCF-7 and MCF-7/ADR cells were cultured in Dulbecco's Modified cultured with RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), antibiotics penicillin (100 IU/mL) and streptomycin (100 mg/mL) were maintained under a fully humidified atmosphere at 37 °C. The MCF-7/ADR cells were grown in the continuous presence of 750 ng/mL of DOX to maintain the ADR-phenotype.

SCID female mice (5–6 weeks old, 18 ± 2 g) were provided by Vital Laboratory Animal Center (Beijing, China). Mice were fed in our specific-pathogen-free facility at the temperature of 24 °C. The animal experiments were conducted in accordance with the animal experiment guidelines approved by Tianjin Medical University.

2.2. Synthesis of MSNs

MSNs were synthesized according to the reference with slight modifications [34]. Firstly, 1.0 g CTAB and 280 mg NaOH were dissolved in 480 mL deionized water and the temperature of the solution was adjusted to 80 °C. Then, 7 mL of the silica precursor, TEOS, was added dropwise at a rate of 1 mL/min using a peristaltic pump. The reaction mixture was vigorously stirred at 80 °C for 2 h, leading to the formation particles. The resulting solid was centrifuged (8000 r/min, 20 min), washed thoroughly with water and methanol for several times and dried in vacuum. To remove the structure-directing agent (CTAB), the prepared sample (0.7 g) was redispersed in the mixture of ethanol (70 mL) and concentrated HCl (0.70 mL, 37.2%). After ethanol extraction for 16 h, the product was collected and washed using deionized water at least three times to obtain MSNs.

2.3. TPGS surface modification

In a typical surface modification process [16,35], 20 mg of MSNs and excessive (~100 mg) TPGS were dispersed in 10 mL of cyclohexane in a 50-mL flask under 10 min sonication, then 20 mL of deionized water was added and the mixture was sonicated for another 10 min. After that, the flask was delivered into a 70 °C water bath under stirring to evaporate the cyclohexane. TPGS modified MSNs (MSNs-TPGS) can be obtained by centrifugation, washed twice with deionized water, and finally dried by lyophilization.

2.4. Synthesis of DOX@MSNs-TPGS

100 mg MSNs-TPGS and 25 mg DOX were dispersed in 10 mL of DMF by sonication. The mixture was stirred at room temperature in the dark for 24 h. The resulting crude product was centrifuged (8000 r/min, 10 min), washed thoroughly with a DOX solution, then with water until the upper liquid is colorless, and dried in vacuum.

To determine the in vivo targeting of MSNs-TPGS, fluorescent dye Cy5.5 was taken as a model fluorescent probe. To formulate Cy5.5 labeled MSNs-TPGS, 50 μL of the Cy5.5 dye dissolved in ethanol (1 mg/mL) was added instead of drugs and went through the same way for the preparation of DOX@MSNs-TPGS.

2.5. Characterizations

The particle size, polydispersity of nanoparticles were determined at 25 °C by DLS. The morphology of MSNs-TPGS was observed using TEM. A drop of the sample was deposited on a carbon coated copper grid to form a film. Before the film was dried, a drop of the dyed solution was added to the film and re-stained with 2% phosphotungstic acid. The grids were dried at room temperature and the samples were checked under TEM.

2.6. Determination of encapsulation efficiency and loading efficiency

To assess the drug encapsulation efficiency (EE) and loading efficiency (LE), a predetermined aliquot of DOX@MSNs-TPGS were first eluted in order to remove the extra free DOX. The DOX concentrations in the nanoparticles before and after elution were evaluated using reverse phase high performance liquid chromatography (RP-HPLC). The percentage of DOX entrapped in the nanoparticles was calculated as follows [36]:

$$LE(\%) = (\text{amount of the drug in nanoparticles} / \text{amount of the feeding material and drug}) \times 100\%.$$

$$EE(\%) = (\text{amount of the drug in nanoparticles} / \text{amount of the feeding drug}) \times 100\%.$$