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Polymerizable disulfide paclitaxel prodrug for controlled drug delivery

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ABSTRACT

A polymerizable disulfide paclitaxel (PTX) prodrug was synthesized by the consequential esterification reactions of 3,3′-dithiodipropionic acid (DTPA), a disulfide compound containing two active carboxyl groups, with 2-hydroxyethyl methacrylate (HEMA) and PTX. The structure of the prodrug was confirmed by ^{1}H NMR characterization. Then, the polymerizable prodrug was copolymerized with poly(ethylene glycol) methyl ether methacrylate (PEGMEA) to obtain a copolymer with hydrophilic PEG side chains and PTX covalently linked onto the backbone via disulfide bonds. The loading content of PTX was 23%. In aqueous solution, this copolymer prodrug could self-assemble into micelles, with hydrophobic PTX as the cores and hydrophilic PEG-segment as the shells. In vitro cell assay demonstrated that this copolymer prodrug showed more apparent cytotoxicity to cancer cells than to human normal cells. After incubation for 48 h, the cell viability of HEK-293 cells (human embryo kidney cells) at 0.1 μg/mL PTX still remained more than 90%, however, that of HeLa cells (human cervical cancer cells) decreased to 52%.

1. Introduction

Chemotherapy, as an indispensable and important strategy in the comprehensive treatment of cancer, has drawn increasing attentions [1–4]. Paclitaxel (PTX) has been proved to be a potent drug for treating a variety of cancers, such as metastatic breast cancer and ovarian cancer [5]. However, the poor water-solubility and non-distinctive cytotoxicity to cancer cells and normal cells greatly hindered its administration dosage and application scope [6–9]. Thus, it's highly required to develop a convenient and safe controlled drug delivery system (CDDS) to maximize the therapeutic efficacy at tumor sites and minimize the side effects of PTX [10–13].

Up to date, a variety of PTX loaded CDDSs, based on pH, thermal, light, or ion strength responsive release mechanisms, have been reported [14]. Many materials, such as liposomes [15], mesoporous silica nanoparticles (MSNs) [16], block copolymer [17], and dendrimers [18], have been utilized as the drug carriers. However, most of these CDDSs loaded PTX by non-covalent interactions. The general routine entraps the drug with the hydrophobic core and function the vehicles with the hydrophilic shell and targeting moieties ensuring the prodrug to release the drug cancer cells. But the burst release of the loaded PTX was inevitable once in the medium [19], due to the weak interaction between the drug and the carrier. Furthermore, the differences of pH, temperature, or light between the lesion sites and the normal tissues were tiny, for example, the pH and temperature of the tumor tissues were approximately 0.5 lower and 1.0 °C higher than those of the normal tissues, respectively. Thus, external complementary assistants were often requisite to enlarge the circumstance differences between the lesion locations and the normal tissues. It's still a challenging work to explore a more efficient and sensitive CDDS for PTX.

Recently, a disulfide bond has attracted growing attentions due to its highly sensitive responsibility [20,21]. A disulfide bond could be readily broken in response to thiol compounds through the thiol–disulfide exchange reaction [22,23]. Only 2 to 3 orders of higher level of glutathione (GSH) tripeptide (approximately 2–10 mM) than the extracellular fluids (approximately 2–20 μM) is enough to induce the disassembly and release of drug in the cytosol [24]. It has been found that the concentration of GSH, is 7-fold higher in human tumor cells than that in normal cells [25]. Thus, a disulfide bond would be stable in the blood circulation and normal tissues, and be broken in tumor tissues [26,27]. The S–S linkage has been widely explored in the design of smart drug vehicles for its redox-responsive property [23]. For example, Ojima et al. have covalently loaded taxoid onto single-walled carbon nanotubes via a disulfide linker, and found that this CDDS showed specific cytotoxicity to L1210FR leukemia cell line [28]. Previously, our group has covalently loaded PTX onto copolymer backbone, and found that this CDDS showed apparent cytotoxicity to OS-RC-2 kidney tumor cells and low cytotoxicity to macrophage human normal cells [29]. However, to our knowledge, all the CDDSs covalently loaded drug via disulfide bonds were constructed by functionalizing carriers firstly, and then linking drug to the carriers.

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Herein, a new strategy to construct a CDDS covalently loaded PTX via disulfide bonds was reported. Firstly, a polymerizable PTX prodrug containing a disulfide bond was synthesized by two sequent esterification reactions of 3,3′-dithiodipropionic acid (DTPA) with 2-hydroxyethyl methacrylate (HEMA) and PTX. Then, the polymerizable PTX prodrug was copolymerized with poly(ethylene glycol) methyl ether methacrylate (PEGMEA) to obtain a copolymer with hydrophilic PEG side chains and PTX covalently linked onto the backbone via disulfide bonds (see Fig. 1). The loading content of PTX could reach up to 23%. In aqueous solution, this copolymer could self-assemble into micelles, with hydrophobic PTX as the cores and hydrophilic PEG-segment as the shells. The mean diameter of the micelles evaluated by dynamic light scattering (DLS) was approximately 210 nm. In vitro cell assays were performed to evaluate the cytotoxicity of the CDDS to human embryo kidney cells (HEK-293) and HeLa human cervical carcinoma cells.

2. Experimental section

2.1. Materials

Paclitaxel (PTX) was purchased from Beijing Huafeng United Technology Co., Ltd. Poly(ethylene glycol) methyl ether methacrylate (PEGMEA, *M*n = 475) and 2-hydroxyethyl methacrylate (HEMA, 98%) were purchased from Aladdin and purified by passing through a neutral alumina column to remove the inhibitor. 2,2′-Azobis(isobutyronitrile) (AIBN) was purchased from Tianjin Guangfu Fine Chemical Research Institute, and purified by dissolving in chloroform and precipitating in methanol. 3,3′-Dithiodipropionic acid (DTPA, 99%), *N*,*N*′-diisopropylcarbodiimide (DIC, 98%) and dimethylaminopyridine (DMAP, 99%) were purchased from Aldrich. Tetrahydrofuran (THF), hexane, ethyl acetate, methylbenzene, dichloromethane, and diethyl ether were purchased from Shanghai Medpep Co., Ltd. Cell counting kit-8 (CCK-8) was purchased from Dojindo Molecular Technologies, Inc.

2.2. Synthesis of polymerizable PTX prodrug 2

Compound **1** (DTPA-HEMA) was synthesized according to the former report [30]. Typically, DTPA (4.4 g, 20 mmol), HEMA (0.6 mL, 5 mmol) and DMAP (0.05 g, 0.4 mmol) were dissolved in THF (50 mL). Then, DIC (0.5 mL, 3.2 mmol) was added to the mixture, and the solution was stirred at room temperature overnight. After reaction, the mixture was concentrated and purified by flash column chromatography on silica using hexane/ethyl acetate (1:1, v/v) as the eluent, to give the desired product **1** (1.17 g, yield: 72%).

Then, PTX (1.0 g, 2.4 mmol), DMAP (0.024 g, 0.2 mmol) and DTPA–HEMA (0.78 g, 2.4 mmol) were dissolved in dichloromethane (70 mL). After adding DIC (23 μL, 0.16 mmol), the solution was stirred at room temperature overnight. After reaction, the mixture was concentrated and purified by flash column chromatography on silica using hexane/ethyl acetate (1:1, v/v) as the eluent, to give the desired polymerizable PTX prodrug **2** (PTX–DTPA–HEMA, 1.328 g, yield: 48%).

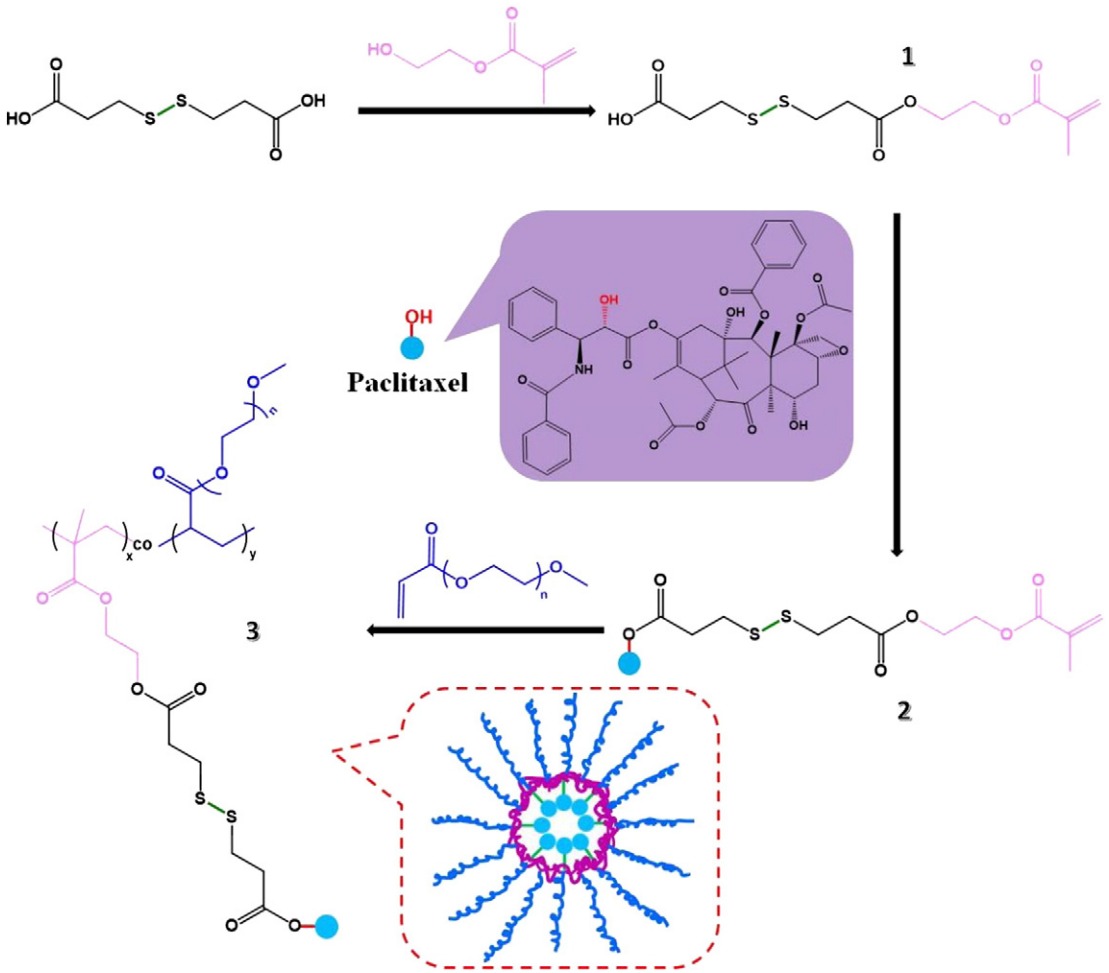

Fig. 1. Schematic illustration of the synthesis of the copolymer.