Well-defined polymer-drug conjugate engineered with redox and pH-sensitive release mechanism for efficient delivery of paclitaxel

Shixian Lv [a,c], Zhaohui Tang [a], Dawei Zhang [a], Wantong Song [a], Mingqiang Li [a,c], Jian Lin [a,c], Huaiyu Liu [b], Xuesi Chen [a,*]

[a] Key Laboratory of Polymer Ecomaterials, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, PR China
[b] Laboratory Animal Center, Jilin University, Changchun 130012, PR China
[c] University of Chinese Academy of Sciences, Beijing 100039, PR China

ARTICLE INFO

ABSTRACT

The synthesis of polymer–drug conjugate (PDC) capable of convenient preparation and controlled release of therapeutic agents is still an urgent requirement in drug delivery field. Herein, we develop a novel anti-cancer PDC engineered with side groups of disulfide and ester bonds for on-demand delivery of paclitaxel (PTX) with redox and pH dual sensitive behaviors. A simple polymer, 3,3′-dithiodipropionic acid functionalized poly(ethylene glycol)-*b*-poly(ʟ-lysine) (mPEG-*b*-P(LL-DTPA)), was synthesized and PTX was directly conjugated to the carboxyl groups of mPEG-*b*-P(LL-DTPA) to obtain the disulfide-containing polymer–PTX conjugate (P(L-SS-PTX)). Another structural similar polymer–PTX conjugate without disulfide bonds (P(L-PTX)) was also prepared to verify the function of disulfide linkages. The P(L-SS-PTX) micelles showed rapid drug release under tumor-relevant reductive conditions as designed. Interestingly, the PTX release from P(L-SS-PTX) micelles could also be promoted by the increased acidity (pH $\approx$ 5). *In vitro* cytotoxicity study showed that the P(L-SS-PTX) micelles exhibited significantly enhanced cytotoxicity against a variety of tumor cells compared to the non-sensitive P(L-PTX) micelles. The *in vivo* studies on B16F1 melanoma bearing C57BL/6 mice demonstrated the superior antitumor activity of P(L-SS-PTX) over both free PTX and P(L-PTX). This dual-sensitive prodrug provides a useful strategy for anti-tumor drug delivery.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Nano-scaled drug delivery systems based on amphiphilic polymers have been attracting great attention in cancer therapy over recent decades [1–5]. The most powerful antitumor drugs often have great limitations in clinical use, such as poor water solubility, fast degradation under physiological environment, non-specific drug distribution in the body, rapid clearance by reticuloendothelial system (RES) and severe side effects. On the contrary, polymeric nanomedicines usually possess prolonged blood circulation and reduced non-specific uptake owing to their surface modifications (*e.g.*, stealth and charge modification), enhanced tumor accumulation *via* the Enhanced Permeability and Retention (EPR) effect, and decreased inherent toxicity [6–8]. In addition, through appropriate structure design of the synthetic polymers applied for micelle formation, polymeric micelles can obtain versatile functions, such as high drug loading capacity, favorable size distribution, excellent biocompatibility, enhanced *in vivo* stability, and controllable drug release, which make them more suitable for their intended use [1,6,9].

Among many kinds of nanocarriers, polymer–drug conjugates (PDCs) have been extensively studied as an effective type of drug delivery system due to their unique properties [10–13]. Compared to other types of nanomedicines which encapsulate drugs through electrostatic or hydrophobic interactions, the drug molecules within PDCs were covalently bonded to polymer chains through the linkages which might be cleaved to release pharmacologically active moieties under certain conditions. As a consequence, PDCs typically possess excellent stability against environmental changes and can overcome the shortcomings of other types of nanomedicines, such as rapid diffusion of encapsulated drugs from the nanocarriers during blood circulation caused by dynamic instability [14]. Accordingly, a few PDCs fabricated by some polymers with simple and well-defined structure (*e.g.*, polyethylene glycol-*b*-poly(aspartic acid), poly-ʟ-glutamic acid and functionalized polyethylene glycol) and several frequently-used antitumor drugs (*e.g.*, taxanes, platinum anticancer drugs, camptothecin and its analogs), have come into clinical trials [15–19]. In most of these PDCs, drug moieties are mainly conveniently conjugated to polymer scaffolds through ester bonds which exhibited outstanding stability

during blood circulation. However, such linkages might be too stable even in the tumor cells to release antitumor drugs effectively. The insufficient drug release may impair the overall anticancer efficiency of these PDCs and hamper their final applications into human bodies [20].

In addition to the excellent extracellular stability, a temporally and spatially controlled drug release in response to the triggers at the target sites is still required for an ideal PDC. Due to the abnormal physiological conditions of the tumors (*e.g.*, glutathione, pH, temperature, enzyme and other bioactive ligands, *etc.*), a batch of PDCs functionalized with enzymatically sensitive linkages [21], pH-sensitive ester or amido bonds [22–25], redox-sensitive disulfide or diselenide bonds [26–28], have been developed for tumor microenvironment-responsive drug delivery, and achieved improved stability and better therapeutic effects. For example, the significant concentration difference of glutathione (GSH) between the extracellular (~2.0 to 20.0 μM) and intracellular (~10 mM) environment has been widely utilized as an ideal trigger for the redox-responsive delivery systems [29,30]. Li et al. reported a polyethylene glycol (PEG)-camptothecin (CPT) prodrug engineered with disulfide linker which could selectively release the drug moieties under tumor reductive conditions [20]. Wang et al. reported a camptothecin (CPT)-conjugated, core-cross-linked (CCL) micelles with redox-responsive disulfide bonds, showing enhanced stability under physiological conditions and rapid drug release in reductive environment [31]. However, even if great progress has been made in this researching field, many PDCs still have some limitations, such as inefficient drug release and complicated synthetic routes. The development of stimuli-responsive PDCs with simple synthesis and well-defined structure is still an urgent need.

Taking advantage of the huge differences between the extracellular and intracellular environment, we develop a novel PDC engineered with disulfide bonds as a stimuli-responsive delivery system for PTX. A polypeptide-based copolymer, 3,3′-dithiodipropionic acid functionalized methoxy poly(ethy1ene glycol)-*b*-poly(L-lysine) (mPEG-*b*-P(LL-DTPA)) was synthesized as the polymeric scaffold. Paclitaxel (PTX), a frequently-used antimicrotubule agent with potent antitumour activity and extremely poor water solubility, was directly conjugated to mPEG-*b*-P(LL-DTPA) through simple ester condensation reaction to obtain the disulfide-containing polymer–PTX conjugate (P(L-SS-PTX)). Another structural similar polymer–PTX conjugate (P(L-PTX)) without disulfide bond was also prepared as a control (Scheme 1).
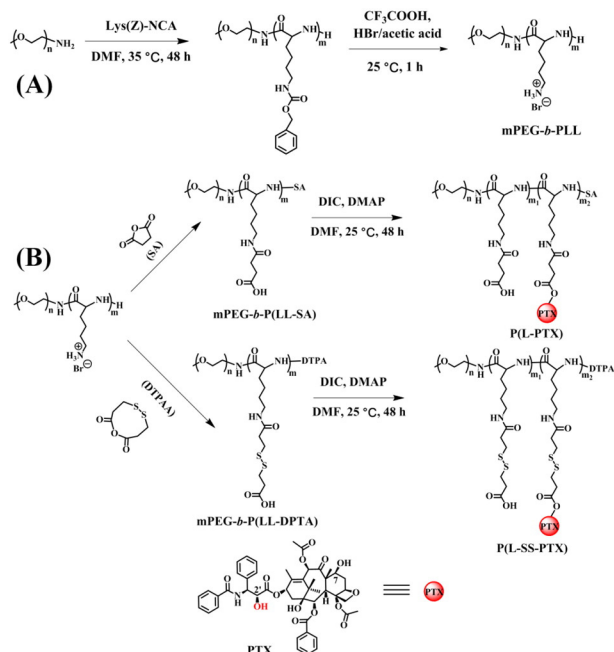
Scheme 1. Synthesis pathway of mPEG-*b*-PLL (A), P(L-PTX) and P(L-SS-PTX) (B).

In the present work, the preparation and characterization of P(L-SS-PTX) and P(L-PTX) were presented. The drug release behaviors, *in vitro* cytotoxicities, and the *in vivo* antitumor activities of the designed PDCs were also performed to investigate the effects of disulfide linkages.

2. Materials and methods

2.1. Materials

Poly(ethylene glycol) monomethyl ether (mPEG, M_n = 5000) and 3,3′-dithiodipropionic acid (DTPA) were purchased from Aldrich and used as received. N^ε-benzyloxycarbony-L-lysine (H-Lys(Z)-OH) was purchased from GL Biochem Co. Ltd. (Shanghai, China). Amino-terminated poly (ethylene glycol) methyl ether (mPEG-NH₂) was prepared according to our previous work [32]. N^ε-benzyloxycarbonyl-L-lysine-N-carboxyanhydride (Lys(Z)-NCA) was synthesized according to the literature [33]. 3,3′-Dithiodipropionic acid anhydride (DTPAA) was prepared according to the literature [34]. Paclitaxel (PTX) was purchased from Beijing Huafeng United Technology Corporation. *N,N*-dimetylformamide (DMF) was stored over calcium hydride (CaH₂) and purified by vacuum distillation with CaH₂. 3-(4,5-Dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) were purchased from Sigma. Fluorescein isothiocyanate (FITC) was purchased from Aladdin. Succinic anhydride and other reagents and solvents were purchased from Sinopharm Chemical Reagent Co. Ltd. and used without further purification.

2.2. Characterizations

The ¹H NMR, zeta potential, dynamic laser scattering (DLS), critical micelle concentration (CMC), and transmission electron microscopy (TEM) measurements were performed as our previous study [35]. Molecular weight distributions (polydispersity index, PDI = M_w/M_n) of the copolymers were determined by gel permeation chromatography (GPC) using the same test condition as our previous work [35].

2.3. Synthesis of mPEG-b-PLL block copolymer

The detailed synthesis procedures of methoxy poly(ethylene glycol)-*b*-poly(L-lysine) (mPEG-*b*-PLL) were described in the Supplementary information.

2.4. Synthesis of mPEG-b-P(LL-DTPA)

3,3′-Dithiodipropionic acid functionalized mPEG-*b*-PLL (mPEG-*b*-P(LL-DTPA)) was synthesized by a ring-opening reaction of mPEG-*b*-PLL with cyclic anhydride DTPAA. In brief, mPEG-*b*-PLL (2.31 g, 0.3 mmol) and DTPAA (1.58 g, 15.6 mmol) were dissolved in dry DMF (20 mL), then triethylamine (TEA) (1.58 g, 15.6 mmol) was added into the solution. The reaction mixture was maintained at 35 °C for 4 h. The mPEG-*b*-P(LL-DTPA) crude product was obtained by repeating precipitation into excess diethyl ether. The crude product was dissolved in DMF and dialyzed (MWCO 3500 Da) against distilled water to remove small molecules. The mPEG-*b*-P(LL-DTPA) flaxen powder was obtained after lyophilization. ¹H NMR of mPEG-*b*-P(LL-DTPA) was measured using DMSO-d_6 as a solvent.

2.5. Synthesis of mPEG-b-P(LL-SA)

The synthesis route of succinic acid functionalized mPEG-*b*-PLL (mPEG-*b*-P(LL-SA)) was similar to that of mPEG-*b*-P(LL-DTPA). Briefly, mPEG-*b*-PLL (2.31 g, 0.3 mmol) and succinic anhydride (SA, 1.95 g, 19.5 mmol) were dissolved in dry DMF (20 mL). Then triethylamine (TEA) (1.58 g, 15.6 mmol) was added into the solution and the mixture was maintained at 35 °C for 4 h. The mPEG-*b*-P(LL-SA) crude product