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Polypeptide-based combination of paclitaxel and cisplatin for enhanced chemotherapy efficacy and reduced side-effects

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ABSTRACT

A novel methoxy poly(ethylene glycol)-*b*-poly(L-glutamic acid)-*b*-poly(L-phenylalanine) (mPEG-*b*-P(Glu)-*b*-P(Phe)) triblock copolymer was prepared and explored as a micelle carrier for the co-delivery of paclitaxel (PTX) and cisplatin (*cis*-diamminedichlo-platinum, CDDP). PTX and CDDP were loaded inside the hydrophobic P(Phe) inner core and chelated to the middle P(Glu) shell, respectively, while mPEG provided the outer corona for prolonged circulation. An in vitro release profile of the PTX + CDDP-loaded micelles showed that the CDDP chelation cross-link prevented an initial burst release of PTX. The PTX + CDDP-loaded micelles exhibited a high synergism effect in the inhibition of A549 human lung cancer cell line proliferation over 72 h incubation. For the in vivo treatment of xenograft human lung tumor, the PTX + CDDP-loaded micelles displayed an obvious tumor inhibiting effect with a 83.1% tumor suppression rate (TSR%), which was significantly higher than that of a free drug combination or micelles with a single drug. In addition, more importantly, the enhanced anti-tumor efficacy of the PTX + CDDP-loaded micelles came with reduced side-effects. No obvious body weight loss occurred during the treatment of A549 tumor-bearing mice with the PTX + CDDP-loaded micelles. Thus, the polypeptide-based combination of PTX and CDDP may provide useful guidance for effective and safe cancer chemotherapy.

1. Introduction

In small-molecule-based chemotherapy, the use of a single agent often fails to achieve complete cancer remission due to the rapid development of drug resistance in tumor cells [1]. Accordingly, a combination of multiple non-cross-resistant anticancer agents has been widely applied clinically [2–4]. Combination chemotherapy offers several benefits. Firstly, applying multiple drugs with different molecular targets can raise the genetic barriers and delay the cancer adaption process. Secondly, multiple drugs targeting the same cellular pathways can function synergistically, giving higher therapeutic efficacy and target selectivity [5–7]. Paclitaxel (PTX) is a representative microtubule-stabilizing chemotherapy drug. Cisplatin (CDDP) is one of the most widely used DNA-modifying chemotherapy drugs. The PTX + CDDP combination of free drugs has shown a good synergism effect against a wide range of cancer cell lines due to the different mechanisms by which PTX and CDDP act [8,9]. Nowadays the PTX + CDDP combination of free drugs has become the first-line chemotherapy for advanced non-small-cell lung cancer [10], ovarian cancer [11], advanced gastric cancer [12,13], advanced breast cancer [14,15] and metastatic esophageal cancer [16].

However, drug combination does not just involve putting things together. One of the most important dose-limiting toxicities of CDDP is nephrotoxicity, while increased nephrotoxicity was observed when PTX was co-administered in gynecological cancers as compared to CDDP alone [17]. In another study, the combination of PTX and CDDP showed an effective clinical response in the treatment of advanced transitional urothelium carcinoma; however, there was an increased toxic effect, and therefore it could not be applied to patients with poor performance status or those over 70 years old [18]. In a phase III study on patients with advanced ovarian cancer, the PTX + CDDP free drug combination regimen did not obviously improve the survival times but did increase hospitalizations and hematological toxicities [19]. Therefore, the additional clinical benefit gained from the use of PTX + CDDP free

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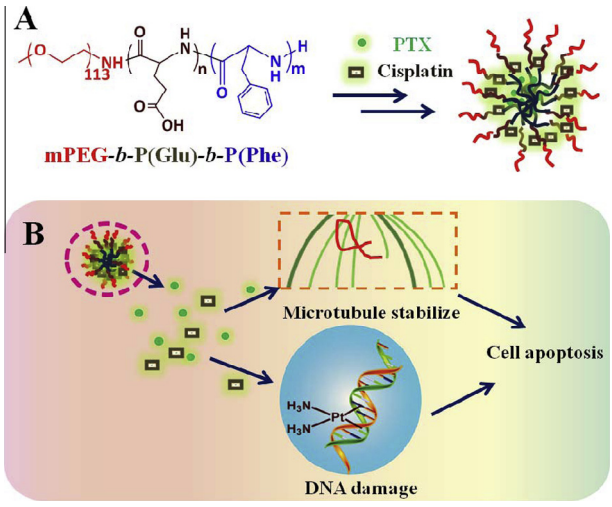

Scheme 1. (A) Preparation of the PTX and CDDP dual-drug-loaded micelles via entrapping into the mPEG-*b*-P(Glu)-*b*-P(Phe) triblock copolymer. (B) Release of PTX and CDDP along with microtubule stabilization and DNA damage, resulting in cell apoptosis.

drug combination chemotherapy was discounted because of the increased toxicity and side-effects.

Advances in nanotechnology have opened up unprecedented opportunities for controlled drug delivery and novel combination strategies. Nanocarriers can remarkably suppress the premature degradation and non-specific interactions of the small molecule drugs with normal tissues, and increase the accumulation of drugs in tumor tissues through the enhanced permeability and retention (EPR) effect [20,21]. Therefore, nanoparticle-based drug delivery systems are characterized by enhanced efficacy and reduced side-effects. These features are heritable in nanocarrier-based combination drug delivery systems [22,23]. For instance, Wang et al. observed enhanced anti-tumor efficacy with reduced side-effects by co-delivery of doxorubicin and PTX with amphiphilic methoxy PEG-PLGA copolymer nanoparticles [24]. In an attempt to co-encapsulate doxorubicin and cyclosporin A in polyalkylcyanoacrylate nanoparticles, the combined nanoparticle formula showed improved growth inhibition efficacy in a resistant cell culture line [25].

Until now, few attempts have been made to co-encapsulate PTX and CDDP in a single nanocarrier and to solve the problem of additive side-effects [26]. The challenge of entrapment of these two drugs into one system might be due to the hydrophobic nature of PTX and the hydrophilic metal complex nature of CDDP. Xiao et al. attempted to co-deliver these two drugs via a biodegradable amphiphilic copolymer with a strategy of conjugating PTX and CDDP (IV) to the copolymer and then co-assembling them together. Although reduced synergistic toxicity and enhanced anti-tumor efficacy were observed, the effective drug structure of the prodrug is unclear [27]. In this study, we aimed to co-deliver PTX and CDDP in one nanocarrier while keeping the original form of the drugs intact. To realize this, synthetic polypeptide was utilized for making a new kind of nanocarrier due to its wide biomedical applications [28–30]. Briefly, a methoxy poly(ethylene glycol)-*b*-poly(L-glutamic acid)-*b*-poly(L-phenylalanine) (mPEG-*b*-P(Glu)-*b*-P(Phe)) triblock polypeptide was prepared and explored as the micelle carrier for the co-delivery of PTX and CDDP, with P(Phe) the hydrophobic inner core for PTX entrapment, P(Glu) the middle shell for CDDP chelation and mPEG the outer corona for prolonging the blood circulation time [31]. The release behavior, synergism effect and in vitro and in vivo anti-tumor efficacy of the core–shell–corona dual-drug-loaded micelles were evaluated in detail (Scheme 1).

2. Materials and methods

2.1. Materials

γ-Benzyl-L-glutamate-*N*-carboxyanhydride (BLG-NCA) and L-phenylalanine *N*-carboxyanhydride (Phe-NCA) were synthesized as described in our previous work [32]. α-Methoxy-ω-amino-poly(ethylene glycol) ($mPEG_{113}$-NH_2) with number average molecular weight (M_n) of 5000 g mol^{-1} was prepared following the reported procedure [33]. *N*,*N*-Dimethylformamide (DMF) was stored over calcium hydride (CaH_2) and purified by vacuum distillation with CaH_2. PTX was purchased from Beijing Huafeng United Technology Corporation, PR China. CDDP was purchased from Shandong Boyuan Chemical Company, PR China. All the other reagents and solvents were purchased from Sinopharm Chemical Reagent Co., Ltd., PR China and used as received.

2.2. Synthesis of mPEG-b-P(Glu)-b-P(Phe) triblock copolymer

mPEG-*b*-P(Glu)-*b*-P(Phe) triblock copolymer was synthesized through the two-step ring-opening polymerization of BLG-NCA and Phe-NCA in DMF using CH_3O-PEG_{113}-NH_2 as the initiator. In brief, BLG-NCA (4 mmol, 1.05 g) was added to a stirred solution of CH_3O-PEG_{113}-NH_2 (0.2 mmol, 1.0 g) in dry DMF (30 ml) at 30 °C. After 36 h, 0.5 ml solution was drawn out, precipitated into diethyl ether, and methoxy poly(ethylene glycol)-*b*-poly(γ-benzyl-L-glutamate) (mPEG-*b*-PBLG) was obtained for proton nuclear magnetic resonance (^{1}H NMR) and gel permeation chromatography (GPC) measurements. Then Phe-NCA (5 mmol, 0.826 g) in 10 ml DMF was added to the reaction mixture, and the reaction was proceeded for an additional 36 h. Methoxy poly(ethylene glycol)-*b*-poly(γ-benzyl-L-glutamate)-*b*-poly(L-phenylalanine) (mPEG-*b*-PBLG-*b*-P(Phe)) was isolated by precipitating several times into diethyl ether. Subsequently, the dried mPEG-*b*-PBLG-*b*-P(Phe) (1 g) copolymer was dissolved in dichloroacetic acid (10 ml) and HBr/acetic acid (33 wt.%, 3 ml) was added for the removal of the γ-benzyl group. After stirring at 30 °C for 1 h, the mixture was precipitated into excessive ice diethyl ether. The precipitate was redissolved in DMF and then dialyzed against distilled water (molecular weight cut-off (MWCO) = 3500 Da), and then freeze-dried to give the final product mPEG-*b*-P(Glu)-*b*-P(Phe). Overall yield was 80.1%.

^{1}H NMR spectra were measured on a Bruker AV 400 NMR spectrometer in trifluoroacetic acid-d (CF_3COOD). Number-average molecular weights, weight-average molecular weights (M_n, M_w) and molecular weight distributions (polydispersity index (PDI) = M_w/M_n) were determined by GPC using a Waters 515 high-performance liquid chromatography (HPLC) pump, with DAWN EOS 18 Angles Laser Light Scattering Instrument and OPTILAB DSP Interferometric Refractometer (Wyatt Technology) as the detector. The eluent was DMF (containing 0.01 M LiBr) at a flow rate of 1.0 ml min^{-1}, with polystyrene with different molecular weights as standard samples.

2.3. Preparation of PTX + CDDP-loaded micelles

PTX and CDDP-loaded micelles were prepared by a two-step method. Firstly, PTX was loaded into the micelles by a dialysis method. mPEG-*b*-P(Glu)-*b*-P(Phe) (200 mg) and PTX (10 mg) were fully dissolved in 10 ml dimethyl sulfoxide (DMSO) at 80 °C, and then 20 ml phosphate buffer (pH 7.4) was added under stirring. The mixture was stirred for another 6 h and then dialyzed against distilled water using a dialysis bag (MWCO = 3500 Da). After centrifugation for 4 min at 5000 rpm, the supernatant solution was filtered through a 0.45 um filter to gain a clear solution, and