Journal of Controlled Release 254 (2017) 23–33

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Paclitaxel dimers assembling nanomedicines for treatment of cervix carcinoma

Qing Pei[a,b], Xiuli Hu[a], Shi Liu[a], Yang Li[a], Zhigang Xie[a,*], Xiabin Jing[a]

[a] State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, 5625 Renmin Street, Changchun, Jilin 130022, PR China

[b] University of Science and Technology of China, Hefei 230026, PR China.

ARTICLE INFO

Keywords:
Paclitaxel
Dimer
Self-assembly
Nanomedicine

ABSTRACT

Poor water solubility and adverse side effects pose a challenge for clinical application of paclitaxel (PTX). In this work, a series of PTX dimers are synthesized by coupling two PTX molecules with dicarboxylic acids. As-synthesized PTX dimers form stable nanoparticles in aqueous solution without using any surfactants or adjuvants, and the solubility of PTX in water increases by 2500-fold compared to that of free PTX. These nanoparticles with high content of PTX are internalized by cancer cells and exhibit comparable cytotoxicity with Taxol. Furthermore, when the PTX dimers are incorporated into methoxypoly(ethylene glycol)$_{2K}$–*block*-poly(D, L-lactide)$_{2K}$ (PEG-PDLLA) micelles, the loading content of PTX dimers is as high as 85 wt%. The formed nanoparticles possess the high stability in biological conditions. Both *in vitro* and *in vivo* experiments show that these (PTX dimer)/PEG-PDLLA formulations possess effective cellular uptake and potent cytotoxicity, and exhibit reduced systemic toxicity and enhanced antitumor efficacy towards human cervical tumor. We believe these PTX dimers-based nanoparticles would be an alternative formulation for PTX, and such drug dimer assembling behaviors could be extended to other therapeutic agents.

1. Introduction

Paclitaxel (PTX) is a widely used anticancer drug for a number of solid tumors, but it has poor solubility in aqueous solution [1–5]. Nanoscale formulation for delivery of PTX is a promising approach to increase the solubility and allow drug accumulation in tumor site by virtue of enhanced permeability and retention (EPR) effect [6–16]. Various polymer-based nanomedicines have been reported for several decades. For example, Abraxane and Genoxol-PM are marketed in several countries now [17–22]. And some nanoparticle formulations for PTX are in clinical trials [17,23,24]. All these nanoscale formulations can improve the therapeutic index of PTX by decreasing systemic toxicity and improving antitumor efficacy. However, PTX loading contents are usually lower than 20 wt%, which leads to the usage of a large quantity of vectors and potentially heterogeneous formulations [25–30]. It remains challenging to develop a simple nanoparticle formulation with exceptionally high PTX content to promote chemotherapeutic efficacy and decrease excipient-associated toxicities.

Self-assembly of drug molecules into nanoparticles is a straightforward method to prepare nanomedicines [31–34]. A pioneering example reported by Couvreur and co-workers is the nanomedicines formed by squalenoylated antitumor drugs [35,36]. Thereafter, several nanomedicines were developed from the derivative of the drug by virtue of various supramolecular interactions [37–45]. For example, Yan et al. reported the small molecule nanomedicines prepared from amphiphilic drug-drug conjugates [46]. Wang and He et al. demonstrated the self-assembled nanomedicines through the disulfide-induced organization [47]. It is worthy to mention that some drug molecules could form nanoparticles by themselves, such as curcumin, 10-hydroxycamptothecin and doxorubicin [41,48]. To date, to our knowledge, rarely PTX-based nanoparticles were reported through self-assembly of suitable derivative of PTX in the absence of surfactants.

Recently, we found the organic dimer is prone to assemble into nanoparticles in aqueous solution [42,49]. We hypothesize that PTX dimer is possible to form the nanomedicines in water. In this work, PTX dimers (abbreviated as PTX_2) with various linkers were synthesized through the condensation chemistry. The self-assembly behaviors of PTX_2 in aqueous solution were investigated in detail. The stabilities of PTX_2-based nanoparticles in physiological conditions were evaluated by size distribution changes and Nile red (NR) loading experiments. The cytotoxicities and antitumor efficacies of PTX nanomedicines were studied by using different cell lines and subcutaneous cervical tumor.

* Corresponding author.
E-mail address: xiez@ciac.ac.cn (Z. Xie).

http://dx.doi.org/10.1016/j.jconrel.2017.03.391
Received 19 January 2017; Received in revised form 1 March 2017; Accepted 26 March 2017
Available online 27 March 2017

2. Materials and methods

2.1. Materials

Paclitaxel (PTX) was purchased from Xian Haoxuan Biological Technology Co., Ltd. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC.HCl, GL Biochem), 4-dimethylaminopyridine (DMAP, Aladdin), proteinase K (Merck) and adipic acid (Aladdin) were used as received. 3,3-dithiodipropionic acid was purchased from Shanghai Sun Chemical Technology Co., Ltd. Succinic acid was purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. Octanedioic acid and azelaic acid was purchased from Shanghai 9 Ding Chemical Technology Co., Ltd. Glutathione (GSH) and Nile red (NR) were both purchased from Shanghai Yuanye Biological Technology Co., Ltd. Chloroform-d ($CDCl_3$) was purchased from Qingdao Tenglong Weibo Technology Co., Ltd. Ultrapure water was prepared from a Milli-Q system (Millipore, USA). Solvents for chemical synthesis were purified by distillation.

2.2. Preparation and characterization

2.2.1. Synthesis of C8

PTX (136 mg, 0.16 mmol) was dissolved in dichloromethane (CH_2Cl_2), and then octanedioic acid (15.5 mg, 0.089 mmol), EDC.HCl (67.1 mg, 0.35 mmol) and DMAP (2.2 mg, 0.018 mmol) were added sequentially. After stirring for 1 h at ambient temperature, additional EDC·HCl (32.1 mg, 0.17 mmol) and DMAP (2.2 mg, 0.018 mmol) were added, and reaction was continued for another 24 h. The reaction product was purified using silica gel column chromatography with CH_2Cl_2 and ethyl acetate to give C8. The synthesis process of C4, C6, C9 and SS was similar to that of C8, so it is not described in detail here. All above reaction yields were > 80%. MS (LTQ) *m/z*: calculated for $C_{98}H_{104}N_2O_{30}$ (C4) $[M - H]^-$ 1788.67, found 1788.3. MS (LTQ) *m/z*: calculated for $C_{100}H_{108}N_2O_{30}$ (C6) $[M - H]^-$ 1816.70, found 1816.4. MS (LTQ) *m/z*: calculated for $C_{102}H_{112}N_2O_{30}$ (C8) $[M - H]^-$ 1844.73, found 1844.4. MS (LTQ) *m/z*: calculated for $C_{103}H_{114}N_2O_{30}$ (C9) $[M - H]^-$ 1858.75, found 1858.4. MS (LTQ) *m/z*: calculated for $C_{100}H_{108}N_2O_{30}S_2$ (SS) $[M - H]^-$ 1880.64, found 1879.9.

2.2.2. Synthesis of PEG–PDLLA

Methoxypoly(ethylene glycol)$_{2K}$–*block*-poly(D, L-lactide)$_{2K}$ (PEG-PDLLA) was synthesized according to the method previously reported by our group [50].

2.2.3. Preparation of PTX_2 NPs

All PTX_2 NPs were fabricated using the nanoprecipitation method. Briefly, 4 mL of PTX_2 (1 mg) solution in tetrahydrofuran (THF) was injected into the 10 mL of distilled water at ambient temperature with vigorous stirring. After evaporating organic solvent, the color of the aqueous solution changed into slight blue while nanoparticles were formed. Unincorporated PTX_2 (precipitate) was removed by centrifugation at 5000 rpm for 5 min. The concentration of PTX_2 NPs in solutions was determined through High Performance Liquid Chromatography (HPLC, Shimadzu, CBM-20A) with a UV–vis detector.

2.2.4. Preparation of NR@PTX_2 NPs

The experiment was similar to the fabrication of PTX_2 NPs. Add PTX_2 and NR to deionized water to form NR@PTX_2 NPs.

2.2.5. Preparation of the M(PTX_2)/M(PTX) and NR@M(PTX_2)

The PEG–PDLLA was used as amphiphilic block copolymer to encapsulate hydrophobic PTX_2 drugs. Briefly, an THF solution of PTX_2/PTX and PEG–PDLLA at specific weight ratios was slowly added into deionized water to form drug-loaded nanoparticles. As for NR@M(PTX_2), just add the THF solution of PTX_2, NR and PEG–PDLLA into deionized water during the preparation of nanoscale formulations using the nanoprecipitation method.

2.2.6. Physicochemical characterization

Proton nuclear magnetic resonance (^{1}H NMR) spectra was recorded on a Bruker AV400 M in $CDCl_3$ at 25 °C. Chemical shifts were given in parts per million from that of tetramethylsilane (TMS) as an internal reference. The mass spectrum (MS) analyses were performed on a LTQ ion trap mass spectrometer (Finnigan, USA) equipped with an electrospray source. Size, size distribution and zeta-potential of the nanoparticles were determined by Malvern Zeta-sizer Nano. The scattering angle was fixed at 90° and the measurement was carried out at 25 °C. The morphology of the nanoparticles was measured by transmission electron microscopy (TEM) performed on a JEOL JEM-1011 electron microscope operating at an acceleration voltage of 100 kV. To prepare specimens for TEM, a drop of nanoparticles solution (0.1 mg mL^{-1}) was deposited onto a copper grid with a carbon coating. The specimens were air-dried and measured at room temperature. It was also measured by scanning electron microscopy (SEM) performed on JEOL JXA-840 under an accelerating voltage of 15 kV. UV–vis absorption spectra was obtained using a Shimadzu UV-2450 PC UV–vis spectrophotometer. Fluorescence intensity tests were performed using Perkin Elmer LS-55 fluorospectrophotometer. The cellular localization was visualized under a confocal laser scanning microscope (CLSM) (Zeiss LSM 700, Zurich, Switzerland). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assays were measured at 490 nm by a microplate reader (BioTek, EXL808). Flow cytometry analysis was performed by a flow cytometer (Beckman, USA) which collected 1×10^4 gated events for each sample.

2.3. In vitro studies

2.3.1. Drug encapsulation

The amount of PTX_2 or PTX encapsulated in the PEG-PDLLA was determined by HPLC using a standard curve method. The measurements were as follows: 1 mg reconstituted M(PTX_2) or M(PTX) powder was dissolved in 10 mL of mobile phase (methanol/acetonitrile/water (HPLC grade) 40/40/20 v/v/v), and then 20 μL of solution was injected into sample loop with a flow rate of 1.0 mL min^{-1}. The PTX_2 with different carbon length exhibited different retention time and UV–vis detection wavelength. HPLC conditions for the five samples were as follows: detection wavelength: C4 230 nm, C6 231 nm, C8 228 nm, C9 231 nm, SS 231 nm; retention time: C4 5.5 min, C6 5.7 min, C8 6.5 min, C9 7.1 min, SS 6.3 min. PTX: detection wavelength: 227 nm; retention time: 3.9 min.

The drug loading content (DLC) and drug loading efficiency (DLE) were calculated by the following Eqs. (1) and (2), respectively:

$$\text{DLC (wt\%)} = \frac{\text{the free drug weight in the nonoparticles}}{\text{the weight of nonoparticles}} \times 100\% \quad (1)$$

$$\text{DLE (wt\%)} = \frac{\text{the free drug weight in the nonoparticles}}{\text{the weight of feeding drug}} \times 100\% \quad (2)$$

2.3.2. Critical aggregation concentration (CAC) and critical micelle concentration (CMC) assays

CAC of PTX_2 NPs and CMC of M(PTX_2) was examined using NR probe according to the method as reported in the previous literature [51,52].

2.3.3. In vitro stability of PTX_2 NPs, M(PTX_2) and M(PTX)

We utilized fetal bovine serum (FBS) to investigate the stability of PTX_2 NPs and M(PTX_2) by detecting the change of their size and size distribution at 37 °C for different time periods. The ratio of FBS and PBS (pH 7.4) was set as 10/90 (v/v).