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Paclitaxel-loaded poly(*N*-vinylpyrrolidone)-*b*-poly(ε-caprolactone) nanoparticles: Preparation and antitumor activity *in vivo*

Zhenshu Zhu [a], Yuan Li [a], Xiaolin Li [b], Rutian Li [b], Zhijun Jia [c], Baorui Liu [b], Wanhua Guo [c], Wei Wu [a], Xiqun Jiang [a,d,*]

[a] *Laboratory of Mesoscopic Chemistry and Department of Polymer Science and Engineering, College of Chemistry and Chemical Engineering, Nanjing University, Nanjing, 210093, PR China*
[b] *Department of Oncology, Medical School of Nanjing University & Clinical Cancer Institute of Nanjing University, Nanjing, 210093, PR China*
[c] *Department of Nuclear Medicine, Affiliated Drum Tower Hospital, Medical School of Nanjing University, Nanjing, 210093, PR China*
[d] *Jiangsu Provincial Laboratory for Nanotechnology, Nanjing University, Nanjing, 210093, PR China*

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ABSTRACT

Paclitaxel (PTX)-loaded poly(*N*-vinylpyrrolidone)-*b*-poly(ε-caprolactone) (PVP-*b*-PCL) nanoparticles with high drug payload were successfully prepared by a modified nano-precipitation method and characterized by transmission electron microscopy (TEM), atomic force microscopy (AFM), dynamic light scattering (DLS) and zeta potential. The satisfactory drug loading content (>25%) and high encapsulation efficiency (>85%) were achieved. The *in vivo* real-time biodistribution of PTX-loaded nanoparticles was investigated using near-infrared fluorescence (NIRF) imaging. The antitumor effect of PTX-loaded nanoparticles was evaluated, both, *in vitro* on three different cancer cell lines and *in vivo* on hepatic H22 tumor bearing mice model via intravenous administration (i.v.). It is found that PTX-loaded nanoparticles exhibit significant superior *in vivo* antitumor effect than the commercially available Taxol® formulation by combining the tumor volumes and survival rates measurement, intravital positron emission tomography and computed tomography (PET/CT) imaging.

1. Introduction

Recently, the use of biocompatible and biodegradable polymer nanoparticles for drug delivery and targeting is one of the most active and clinically needful areas in nanomedicine [1]. These polymer nanoparticles prepared by a self-assembly strategy using amphiphilic block copolymers in aqueous medium are generally comprised of hydrophilic outer shell and hydrophobic inner core, and can incorporate lipophilic drugs into their cores and release the drug in a controlled manner at a later stage, making them the promising carriers for poor water solubility drugs [2,3]. It has been pointed out that polymer nanoparticles can preferentially accumulate in tumor after intravenous (i.v.) administration due to the enhanced permeability and retention (EPR) effect, known as "passive" targeting [4,5]. Although this passive manner of drug delivery omits the targeting group at the surface of polymer nanoparticles, it is quite effective to delivery drugs to the tumor sites. To achieve effective tumor accumulation of polymer nanoparticles through EPR effect, the nanoparticles should stay long time in blood circulation. A number of approaches for prolonging the blood circulation and accumulating in tumor tissues of nanoparticles have been reported, including control of size, surface potential as well as surface hydrophilicity [6,7]. In particular, surface modification with flexible, hydrophilic poly(ethylene glycol) (PEG) can help the nanoparticles to diminish uptake by mononuclear phagocytic system (MPS), resulting in prolonging blood circulation time and improving accumulation in tumor tissues of nanoparticles [8,9]. Although PEG has a number of advantages such as biocompatibility, lack of toxicity, very low immunogenicity and antigenicity, and PEGylated nanoparticles increase circulation longevity, it has been reported that PEGylation does not completely avoid cumulative uptake by MPS cells, and PEGylated nanoparticles cannot mostly remove complement system activation for their corresponding naked nanoparticles [10,11]. Burt et al. employed ^{14}C-labeled poly (ethylene glycol)-*block*-poly(D, L-lactide) (PEG-*b*-PDLLA) micelles to probe the biodistribution in rats. It was found that mPEG segments and PDLLA segments were rapidly excreted in urine [12]. Kiwada et al. indicated that immunocompetence related to PEG accelerated blood clearance phenomenon [13–15]. Kaneda and Tsutsumi et al. reported that PEG showed a relatively high urinary excretion and peripheral distribution volume [16,17]. Besides, the molecular structure of PEG as a general polymeric modifier does also not readily allow the addition of new functions along its chain.

An attractive alternative to PEG as surface modifier and stabilizer is the use of water-soluble poly(*N*-vinylpyrrolidone) (PVP). PVP is a well-known hydrophilic biocompatible polymer and used in many

* Corresponding author. Laboratory of Mesoscopic Chemistry and Department of Polymer Science and Engineering, College of Chemistry and Chemical Engineering, Nanjing University, Nanjing, 210093, PR China. Fax: +86 25 83317761.
E-mail address: jiangx@nju.edu.cn (X. Jiang).

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drug delivery systems, such as polymer conjugates and liposomes [18,19]. It was reported that PVP conjugated with tumor necrosis factor-α (TNF-α) showed longer blood circulation time than PEG conjugated TNF-α [16]. Mayumi et al. showed that PVP-TNF-α conjugation was a more potent antitumor therapeutic agent than PEGylated TNF-α [20]. Torchilin et al. found that PVP modification made liposome long-circulating *in vivo* and decreased liposome accumulation in the liver [18,21]. However, compared with PEGylated polymer nanoparticles, the work on PVP modified biocompatible and biodegradable polymer nanoparticles for drug delivery is limited. Cho et al. reported the preparation of poly(*N*-vinylpyrrolidone)-*block*-poly(ε-caprolactone) (PVP-*b*-PCL) micelles but the drug delivery was not addressed [22]. Garrec and Leroux et al. studied PVP-*b*-PDLLA micelles for antitumor drug encapsulation and delivery [23]. Unfortunately, the paclitaxel (PTX) loading capacity was quite low (5%), which limited their practical effects. Very recently, it was reported that the capability of PVP to prevent protein adsorption was correlated with its coating state and molecule weight [24,25]. Gaucher and Leroux et al. found that the nanoparticles coated by PVP with molecular weight of 2500 and 4800 rapidly removed from blood due to complement components and immunoglobulins adsorption [25]. Therefore, to achieve sufficient antitumor effect, there remains elusive by using PVP modified polymer nanoparticles for delivering antitumor agent although the nanoparticles can load water-insoluble antitumor drug and overcome *in vivo* barriers in some degree.

In the present study, we prepared PTX-loaded PVP-*b*-PCL nanoparticles with satisfactory drug loading content (>25%) and encapsulation efficiency (>85%) by modified nano-precipitation method. Using real-time near-infrared fluorescence (NIRF) imaging for long time investigation, the nanoparticles (about 100 nm) had a reasonable *in vivo* circulation time and tumor accumulation ability as well as less uptake by liver and spleen. The antitumor effect of PTX-loaded nanoparticles was evaluated, both, *in vitro* on three different cancer cell lines and *in vivo* on hepatic H22 tumor bearing mice model via intravenous administration (i.v.). We found that PTX-loaded nanoparticles possess a superior *in vivo* antitumor effect than the commercially available Taxol® formulation by combining the tumor volume and survival rate measurements, intravital positron emission tomography and computed tomography (PET/CT) imaging.

2. Materials and methods

2.1. Materials

Paclitaxel (PTX) was obtained from Meilian Pharm Co., Ltd. (Chongqing, China). Taxol® was purchased from Bristol-Myers Squibb (Princeton, NJ, USA). NIR-797-isothiocyanate was bought from Sigma Chemical Co. *N*-vinylpyrrolidone (98%, Acros) was purified by fractional distillation. ε-caprolactone (ε-CL, Sigma) was dehydrated by CaH_2 at room temperature and distilled under reduced pressure. Male ICR mice (6–8 weeks old and weighing 18–22 g) were purchased from Animal Center of Drum Tower Hospital (Nanjing, China). Human gastric carcinoma cell line BGC 823, human epidermoid carcinoma cell line KB and a murine hepatic H22 cell line were purchased from Shanghai Institute of Cell Biology (Shanghai, China).

2.2. Synthesis of PVP-b-PCL

Hydroxyl-terminated PVP (PVP-OH) was synthesized by employing isopropyl alcohol and 2-mercaptoethanol as chain transfer agents [26]. Briefly, VP (10 g, 90 mmol), AIBN (120 mg, 0.732 mmol) and 2-mercaptoethanol (234 mg, 3 mmol) were dissolved in 60 mL of isopropyl alcohol. The solution was degassed and the polymerization was performed at 80 °C for 12 h. After removal of isopropyl alcohol and 2-mercaptoethanol under reduced pressure, PVP-OH was dissolved in chloroform and subsequently precipitated into an excess amount of diethyl ether. The obtained copolymer was dried in vacuum oven at 80 °C and stored in a desiccator under vacuum.

PVP-*b*-PCL was synthesized by ring-opening polymerization as described elsewhere [27]. A predetermined amount of CL monomer was added into a polymerization tube containing PVP-OH (3.2 g, 1 mmol) and a small amount of stannous octoate (0.1% wt/wt). The tube was then vacuated and meanwhile sealed off. Thereafter, the polymerization was run at 130 °C for 48 h. The crude product was dissolved in chloroform and precipitated into excess diethyl ether twice. The mole ratio of VP to CL in the copolymer was calculated from ^{1}H NMR. Typical ^{1}H NMR spectral data: 4.1 δ, (–$COOCH_2$– in PCL); 3.1–3.2 δ, (–CH_2–CO– into VP ring); 2.3 δ, (–CH_2COO– in PCL); 1.3–2.3 δ, (–CH_2– PVP main chain + –CH_2– into VP ring + PCL methylene groups protons).

2.3. Preparation of PTX-loaded nanoparticles

PTX-loaded nanoparticles were prepared by a modified nano-precipitation method. Briefly, 10 mg of PTX and 20 mg of PVP-*b*-PCL diblock copolymer were dissolved in 0.4 mL of ethanol by heating to 60 °C. To this solution was added 5 mL of hot water (50 °C) quickly. The solution turned into bluish immediately due to the formation of nanoparticles. The remaining ethanol was reduced by rotary vacuum evaporation and the resultant bluish solution was filtrated through 0.22 μm cellulose acetate filter membrane to remove non-incorporated drug crystals as well as copolymer aggregates. Finally, the dispersed solution was lyophilized for further use. Empty nanoparticles were prepared in a similar manner omitting the drug. The lyophilized nanoparticles are redispersed in PBS before use.

2.4. Molecular weight and chemical composition analysis

Molecular weight of PVP-OH was determined by a gel permeation chromatography (GPC) (Waters 515 systems) equipped with Waters 1525 pump, Waters 410 differential refractometer, Waters utrahydrogel™ linear columns. 50/50 water (double-distilled)/methanol (HPLC grade) containing 0.1 mol/L $LiNO_3$ was used as eluent at a flow rate of 0.5 mL/min. PEG standards used as calibrations. The chemical composition of synthesized diblock copolymer was characterized on ^{1}H NMR (Bruker DQX-300) using $CDCl_3$ as the solvent for the sample. The composition of the samples was estimated from the ratio of the peak areas assigned to VP and CL blocks in the NMR spectrum. From the VP/CL molar ratio, the number-average molar mass (M_n) of PVP-*b*-PCL was determined.

2.5. Size, zeta potential and morphology of the nanoparticles

Mean diameter and size distribution of the prepared nanoparticles were determined by dynamic light scattering (DLS) using a Brookhaven BI-9000AT system (Brookhaven Instruments Corporation, USA). Zeta potential of the nanoparticles was measured with Zetaplus (Brookhaven Instruments Corporation, USA). Each sample was diluted to a concentration of 0.05% (wt/v) with filtered water or 0.1 mol/L of NaCl solution in the case of zeta potential examination. Both particle size and zeta potential were the average of triplicate measurements for a single sample.

Morphology examination of the nanoparticles was conducted on transmission electron microscopy (TEM, JEM-100 S, JEOL, Japan). The sample was negative stained with phosphotungstic sodium solution (1% wt/v) before observation. Atomic force microscopy (AFM, SPI3800, Seiko Instruments Inc., Japan) was also employed to study the morphology of nanoparticles. One drop of properly diluted nanoparticle suspension was placed on the surface of a clean silicon wafer and air-dried at room temperature. Then the sample was observed by atomic force microscopy (AFM, SPI3800, Seiko Instruments, Japan) with a 20-μm scanner in tapping mode.