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Characterization, pharmacokinetics and disposition of novel nanoscale preparations of paclitaxel

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ABSTRACT

Polymeric nanoparticles (NPs) have great potential application in achieving targeted delivery of anticancer drugs. Paclitaxel (PTX) loaded NPs were developed using biodegradable methoxy poly (ethylene glycol)–poly (ε-caprolactone) (MPEG–PCL) diblock copolymer by solid dispersion technique without toxic organic solvent. The lyophilized powder has been stored at room temperature for more than six months and still unchanged. PTX-loaded MPEG–PCL nanoparticles (PTX-NPs) displayed that the highest drug loading of PTX was about 25.6% and entrapment efficiency was over 98%, and the optimized average diameter and polydispersity index (PDI) were about 27.6 ± 0.1 nm and 0.05, respectively. Moreover, experimental results shown PTX-NPs had sustained-release effects and its curve fitting followed the Higuchi model. The maximum tolerated dose (MTD) of PTX-NPs after single dose in Balb/c mice was above 80 mg PTX/kg body weight (b.w), which was 2.6-fold higher than that of Taxol® (30 mg PTX/kg b.w). The levels of PTX administrated PTX-NPs had obvious distinction to Taxol® in plasma, liver, spleen, kidneys, lungs, heart and tumor. Especially, the concentration of PTX in tumor administrated PTX-NPs was higher than administration of Taxol®. All results suggested that we had contrived a simple, biodegradable, effective and controllable drug delivery system for paclitaxel.

1. Introduction

Due to poor water solubility of many therapeutic compounds, their applications to clinical research were greatly restricted. Paclitaxel (PTX) is a natural anticancer drug, which is effective in treating ovarian cancer, breast cancer, stomach cancer, lung cancer, thyroid cancer, non-small cell lung cancer, AIDS related Kaposi's sarcoma, etc. (Wall, 1998). However, because of its poor aqueous solubility and low therapeutic index, the clinical applications are extremely limited. Clinical applications of PTX (Taxol®) are usually formulated based on Cremophor EL (527 mg/mL) and ethanol (Vyas and Vittorio, 1995). Unfortunately, serious side effects are associated with alcohol-based solvents and Cremophor EL, such as severe hypersensitivity reactions (Weiss et al., 1990), peripheral neuropathy (Rowinsky et al., 1993), etc., which are not well tolerated for patients (Terwogt et al., 1997).

To date, numerous techniques have been attempted to improve the solubility of PTX, such as self-emulsion (Chu et al., 2009), inclusion compound (Liu et al., 2003; Yigitbasi et al., 2004; Zhang et al., 2005), liposomes (Wu et al., 2006), nanosphere (Gao et al., 2008; Gaucher et al., 2010; Hammady et al., 2009; Miglietta et al., 2000; Mu et al., 2010; Musumeci et al., 2006; Ravi Kumar et al., 2004; Wang et al., 2007; Zhang et al., 1996), nanoparticles (Dong and Feng, 2004; He et al., 2007; Lee et al., 2008; Li et al., 2008; Önyüksel et al., 2009; Yadav et al., 2007; Zhang et al., 2005), etc. Polymeric nanoparticle (NP) is a very promising drug delivery system, which has attracted many researchers' attention. Many papers about copolymer nanoparticles have been published (Chan et al., 2009; Gao et al., 2008; Gaucher et al., 2010; Kim et al., 2001; Matsumura, 2008; Mu et al., 2010; Wang et al., 2007; Zhang et al., 1996), only a limited number of products have been successfully used in clinical research (Kim et al., 2001; Matsumura, 2008). The reasons, apart from the toxicity and cost of materials, ascribe to be that many new technologies or concepts were within academia and were not suitable for scaling up.

Now the greatest challenge in developing cancer drugs is to deliver them to the target tumor tissues with lower systemic toxicity. Because tumor tissues have abnormal blood vessels with large pores, NPs in the range of 10–100 nm can leak into the surrounding tumor tissue, which was known as enhanced permeability and retention (EPR) effect (Maeda et al., 2000). It allowed NPs to passively accumulate in the tumor to reduce the side effects of cancer drugs.

Amphiphilic block copolymers are composed of hydrophobic and hydrophilic segments. In aqueous solution, they can self-assemble to form core-shell micellar nanostructures with hydrophobic segments (core) serving as the nanocontainer of

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hydrophobic compounds and hydrophilic domain (shell) serving as a stabilizing interface of NPs. The size of polymeric micelle is typically in range of 10–100 nm. So far, various preparation approaches such as dialysis (Cha et al., 2009; Gong et al., 2009; Letchford et al., 2009; Sheikh et al., 2009), oil-in-water (Jaromin et al., 2006), solvent evaporation (Chavanpatil et al., 2006; Jackson et al., 2004; Jin et al., 2007; Li et al., 2009; Mo and Lim, 2005; Westedt et al., 2007), co-solvent evaporation (Jette et al., 2004; Misra et al., 2009), freeze-drying method (Fournier et al., 2004), and etc., have been developed. However, many current papers focus exclusively on studies of pharmacy stability (clarify or precipitation), while ignoring the preparation process, especially the chemical stability of drugs or using many toxic organic solvents during the preparation process, these factors led to its difficulty to promote the use of existing technology. Therefore, in the future researchers should pay more attention to the preparation process of NPs. With the development of nanoscience, many hurdles of drug formulations can be overcome in the future.

In present work, the MPEG–PCL diblock copolymers shows a wonderful combination with PTX. This PTX-loaded MPEG–PCL nanoparticles (PTX-NPs) and preparation technology are better than PTX formulations previously reported (Beletsi et al., 2008; Chang and Chu, 2008; Dong et al., 2010; Dong and Feng, 2004, 2007; Duan et al., 2006; Gryparis et al., 2007; Katsikogianni and Avgoustakis, 2006; Kim and Lee, 2001; Ko et al., 2007; Lee et al., 2008; Ngawhirunpat et al., 2009; van Hasselt et al., 2009; Wu et al., 2006; Xiong et al., 2008; Zhang et al., 2005). The PTX-NPs was prepared through solid dispersion technique without organic solvent, and the lyophilized powder without cryoprotector can be easily redispersed in water. PTX-NPs may be an excellent PTX formulation that may enter market in the future.

2. Materials and methods

2.1. Materials

Monomethoxy poly(ethylene glycol) (MPEG, *Mn* = 750, 2000, and 5000, Aldrich, USA), ε-caprolactone (ε-CL, Alfa Aesar, USA), stannous octoate ($Sn(Oct)_2$), Dulbecco's modified Eagle's medium (DMEM), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and Paclitaxel were purchased from Sigma (USA). Dehydrated alcohol, dimethylformamide (DMF), tert-butyl alcohol, dichloromethane (DCM) and petroleum ether were purchased from Kelong Chemicals (Chengdu, China). TAXOL® (Bristol-Myers Squibb, USP). The animals were purchased from the Laboratory Animal Center of Sichuan University (Chengdu, China). The animals were housed at temperature of 20–22 °C, relative humidity of 50–60% and 12 h light-dark cycles. Free access to food and water was allowed. All animals would be in quarantine for a week before treatment. All animals care and experimental procedures were conducted according to Institutional Animal Care and Use guidelines.

2.2. Methods

2.2.1. Synthesis of diblock copolymer

MPEG–PCL copolymers with different molecular weight ratios of MPEG/PCL were synthesized by ring-opening polymerization according to previous report (Wei et al., 2009). The obtained MPEG–PCL copolymers were purified and kept in air-tight bags before further application. The macromolecular weight (*Mn*) was characterized by proton nuclear magnetic resonance (^{1}H NMR) spectroscopy.

2.2.2. Preparation of PTX-NPs

2.2.2.1. Preparation of PTX-NPs with different Mn ratios of MPEG–PCL. At 40 °C, ninety-two milligrams of MPEG–PCL copolymer with different macromolecular weights and eight milligrams of PTX were dissolved in 3 ml of dehydrated alcohol under vigorous stirring. After all contents were dissolved, the solution was evaporated on a rotary evaporator under reduced pressure at 60 °C. When alcohol was evaporated, homogenous coevaporation was obtained. PTX was distributed in polymeric carriers as amorphous form. Then the coevaporation was dissolved in water at 65 °C to create PTX-NPs solution, and the solution was filtered with a 0.22 μm filter to gain a clarified solution. Then the solution was lyophilized in a freeze dry system to receive dried PTX-NPs powder.

2.2.2.2. Preparation of PTX-NPs with different methods. (1) Dialysis method. PTX-NPs were prepared according to the method reported previously (Kim and Lee, 2001): PTX (8 mg) and MPEG–PCL (92 mg) were dissolved in 10 ml DMF. Then the solution was transferred into a dialysis tube (Spectrapore, MWCO3500) to dialyze against distilled water for 24 h. The outer solution was exchanged at appropriate time interval. After 4000 r/min centrifuged, the supernatant solution was filtered with a 0.22 μm filter to gain a clarified solution and then freeze-dried to obtain the resultant drug-loaded powder. The dried powder was rehydrated with water before application.

(2) Solid dispersion technique. The process for the preparation of PTX-NPs was the same as the method of preparation of PTX-NPs with different *Mn* ratios of MPEG–PCL.

(3) Freeze-drying PTX (8 mg) and MPEG–PCL (92 mg) were dissolved in 4 ml tert-butyl alcohol (TBA)/water (v/v, 3:1) mixed solution. The solution was filtered with a 0.22 μm filter to gain a clarified solution and then freeze-dried. The dried power was rehydrated with water before application (Fournier et al., 2004).

2.2.3. Characterization of PTX-NPs

2.2.3.1. Drug loading, entrapment efficiency and yield. The amount of nanoparticles was first dissolved in acetonitrile to destruct its package status and filtered through 0.22 μm filter to obtain a clear solution, then the sample solution was determined at 227 nm using reverse-phase High Performance Liquid Chromatography (RP-HPLC) with a C_{18} column (4.6 mm × 150 mm – 5 μm, Grace Analysis column), with acetonitrile/water (48/52, v/v) as eluent solution. Drug-loading (DL), entrapment efficiency (EE) and yield (Y) were obtained by the following calculation equations:

$$\text{DL}\ \% = \frac{\text{Amount of PTX determined in micelle}}{\text{Amount of PTX determined} + \text{copolymer}} \times 100\% \quad (1)$$

$$\text{EE}\ \% = \frac{\text{Amount of PTX loaded in micelle}}{\text{Amount of PTX in feed}} \times 100\% \quad (2)$$

$$\text{Y}\ \% = \frac{\text{Amount of freeze-dried powder}}{\text{Amount of PTX and copolymer in feed}} \times 100\% \quad (3)$$

The characterization of PTX-NPs such as particle size, zeta potential and polydispersity index (PDI) were characterized by Malvern Nano-ZS 90 laser particle size analyzer after equilibration for 10 min. The stability of the NPs was evaluated based on the *Particulate Matter in injections* of USP Pharmacopoeia, each sample should be inspected without observable foreign and particulate matter in its solution. The morphological characteristics of PTX-NPs were examined by transmission electron microscope (TEM, H-6009IV, Hitachi, Japan): nanoparticles were diluted with distilled water and placed on a copper grid covered with nitrocellulose. The samples were negatively stained with phosphotungstic acid and dried at room temperature.

2.2.3.2. Crystallographic study. Crystallographic assay was performed on PTX powder, blank MPEG–PCL copolymer, physical mixture of PTX and MPEG–PCL, and PTX-NPs freeze-dried powder by PHILIPS X-ray Diffraction (XRD, X'Pert Pro, MPDDY 1291) using