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Self-assembled Micelle Loading Cabazitaxel for therapy of Lung Cancer

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ABSTRACT

Lung cancer is a leading cause of cancer deaths worldwide, chemotherapy has improved overall survival but remains limited at <12 months median overall survival. Cabazitaxel is hopeful to do the same in advanced lung cancer as well as in metastatic prostate cancer. However, its clinical application was restricted due to its high hydrophobicity and severe side effects. To overcome these problems, we developed self-assembled micelle loading cabazitaxel (CBZ-PM) for therapy of lung cancer. The CBZ-PM has high drug loading (10.52%) and encapsulation efficiency (99.30%) with particle size of 28.77 ± 0.52 nm and polydisperse index of 0.114 ± 0.012. The transmission electron microscope image presented its spherical and homogeneous appearance. *In vitro* release profile showed CBZ-PM has a sustained-release behavior. Furthermore, the result of cell proliferation assays proved that CBZ-PM could induce the Lewis lung carcinoma (LLC) cells death through G_2/M arrest more effectively than free CBZ. *In vivo* anti-tumor activity of CBZ-PM was further studied in mice model of LLC. The tumor inhibitory rate of CBZ-PM was more than 50% and the survival time of LLC bearing mice was efficiently prolonged following administration of CBZ-PM. In addition, the immunohistochemical study showed that more apoptosis cells were detected in the tumor tissue of CBZ-PM group than that of the positive control group. All these indicated that CBZ-PM served as a potential anti-lung cancer agent.

1. Introduction

Lung cancer is one of the most commonly diagnosed malignancies with high morbidity and mortality rates (Siegel et al., 2015). Despite recent advances in chemotherapy, which has improved overall survival but remains limited at <12 months median overall survival (Ferlay et al., 2015). To increase the survival rates, great efforts have been made to find chemotherapeutic drug with novel anti-cancer mechanism (Azzoli et al., 2011; Ciuleanu et al., 2012). Taxanes, such as paclitaxel and docetaxel have been recognized by many oncologists as the first-line drug to treat lung cancer (Rowinsky and Eric, 1997; de Weger et al., 2014) However, the success is poor and overall survival has not improved for more than a decade (Lee et al., 2013a). The major reason for poor prognosis of lung cancer is the high chemo-resistance after taxanes treatment (Murthy and Shah, 2007). Hence, it is necessary to explore new drugs aiming at chemo-resistant cancer cells to provide an effective therapeutic strategy for lung cancer.

Cabazitaxel (CBZ, Fig. 1A is its chemical structure) was rationally and specifically designed and selected to be an active anticancer treatment in taxane-resistant tumors, which was the second-generation taxane with superior cytotoxicity compared with docetaxel even in docetaxel-sensitive cell lines (Azarenko et al., 2013; Tsao et al., 2011; Wilson et al., 2015). Notably, in June 2010, CBZ has been approved for the treatment of prostate cancer by the US Food and Drug Administration (Abidi, 2013; Paller and Antonarakis, 2011). Meanwhile, CBZ is hopeful to do the same in advanced lung cancer as well as in metastatic prostate cancer. However, its high hydrophobicity is the bottleneck for clinical application in anti-tumor. Jevtana®, the only commercial dosage form of CBZ, contains large amount of polysorbate 80 and dehydrated ethanol as co-solvents, leading to serious side effects including generalized rash/erythema, hypotension and bronchospasm (Norris et al., 2010). In addition, the conventional formulations of antineoplastic agents, just like Jevtana®, have

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become suboptimal treatment due to rapid clearance, poor tumor-specific accumulation and excessive toxicities. Therefore, it is necessary to develop a water-based formulation of CBZ without any co-solvent aiming at chemo-resistant lung cells to improve therapeutic index and reduce side effects.

Over the past two decades, polymeric micelle (PM) has received intensive attentions in the field of drug delivery systems because it can figure out the above-mentioned problems (Wang et al., 2015). It can not only improve therapeutic efficacy but also reduce unwanted side-effects (Lee et al., 2013b). PM present great therapeutic potential due to its low critical micelle concentration (CMC) in water, prolonged blood circulation time and high accumulation in tumor tissue based on the enhanced permeability and retention (EPR) effect (Maeda, 2010). In addition, the PM has core/shell architecture (Fig. 1B) which is composed of hydrophobic blocks as internal core, providing a loading space for hydrophobic drugs, and hydrophilic blocks as surrounding corona in aqueous medium for preventing micelle from opsonizing and subsequent uptake by mononuclear phagocyte system (MPS) (García et al., 2014). Besides the foregoing superiorities, PM can increase intracellular drug accumulation and overcome drug-resistance issues to some extent through lessening the *P*-glycoprotein efflux effect (Gong et al., 2012; Kapse-Mistry et al., 2014). Taken together, we had the following hypothesis that PEGylated nano-micelle might serve as carriers for CBZ to realize efficient extravasation from fenestrated capillaries to tumor tissues and escape from MPS.

In the present study, we described the preparation and characterization of CBZ loaded in polymeric micelle (CBZ-PM) as drug delivery system for lung cancer treatment. CBZ-PM was fabricated by one-step solid dispersion method and characterized using dynamic light scattering (DLS), transmission electron microscope (TEM), atomic force microscope (AFM), X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FTIR). Besides, the *in vitro* release behavior of CBZ-PM was investigated using a modified dialysis method. To test the activity of encapsulated CBZ, cell proliferation assays and cell cycle analysis were conducted on Lewis lung carcinoma cells. Subsequently, subcutaneous Lewis lung carcinoma mouse models were established and used to evaluate anti-tumor activity of CBZ-PM. Finally, our findings indicated that CBZ-PM showed improved anti-tumor activity both *in vitro* and *in vivo*, and had potential applications in therapy of pulmonary carcinoma.

2. Materials and methods

2.1. Materials, cell lines, and animals

Monomethoxy polyethylene glycol (mPEG, M_w = 2000 Da), ε-caprolactone (ε-CL), stannous octoate ($Sn(Oct)_2$) were purchased from Sigma–Aldrich Chemical Corp. (Shanghai, China); Cabazitaxel was supplied by the Shanghai Institute of Pharmaceutical Industry (Shanghai, China). Dulbecco's Modified Eagle Medium (DMEM) High Glucose was purchased from HyClone Thermo Scientific. Fetal Bovine Serum (FBS) was purchased from Gibco-Life Technologies. Penicillin/streptomycin and 0.25% (w/v) trypsin—0.1% (w/v) Ethylene Diamine Tetraacetic Acid (EDTA) were purchased from Solarbio (Beijing Solarbio Science and Technology, China). 3-(4, 5-dimethyl-thiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) and dimethylsulfoxide (DMSO) were purchased from sigma (USA). Culture flasks and dishes were from Corning (Corning, NY, USA). HPLC-grade acetonitrile was purchased from Anaqua Chemical Supply (Houston, TX, USA). Ethanol absolute, Tween 80, ammonium acetate of AR grade and spectrographic grade potassium bromide (KBr) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All other chemicals were of analytical reagent grade and purchased from commercial sources.

The mouse Lewis lung carcinoma (LLC) cell line was purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). This cell line was established from the lung of a C57BL mouse bearing a tumor resulting from an implantation of primary Lewis lung carcinoma. The cells were cultured in DMEM medium, completed by adding 10% FBS and 1% penicillin–streptomycin, at 37 °C in a humidified incubator in 5% CO_2.

Six to eight week old C57BL/6 mice were purchased from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China.) All mice had free access to food and water and they were housed at controlled temperature of 20–22 °C with relative humidity of 50–60% and 12 h light and dark cycles. All experiments were carried out in accordance with the guidelines of the local animal welfare committee.

2.2. Synthesis and characterization of the mPEG-PCL

The mPEG-PCL di-block copolymer was synthesized by the method reported before (Liggins and Burt, 2002). In brief, it was synthesized by ring opening polymerization of ε-CL in the

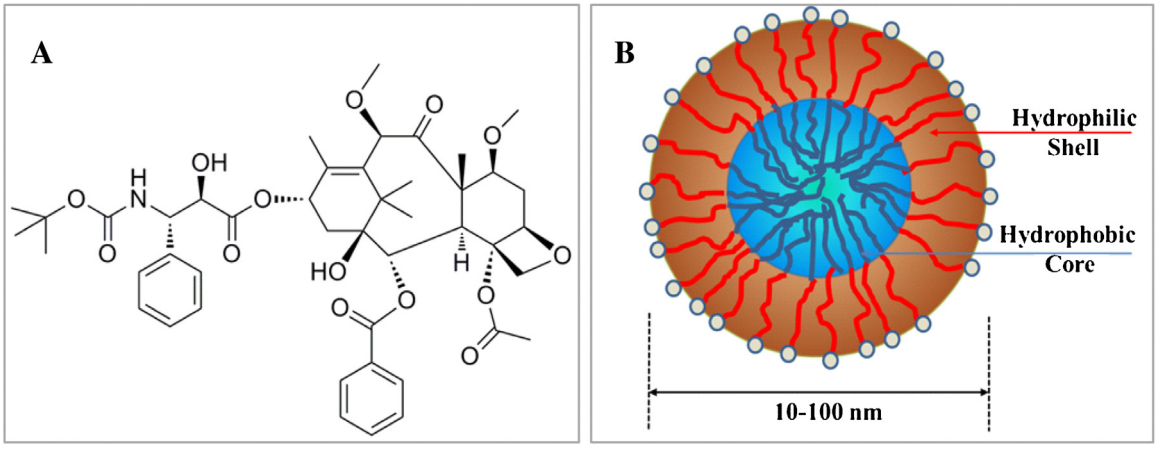

Fig. 1. (A) The chemical structure of CBZ. (B) The schematic representation of core-shell structure of polymeric micelle.