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Preparation and evaluation of teniposide-loaded polymeric micelles for breast cancer therapy

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ABSTRACT

Self-assembled polymeric micelles have been widely applied in anticancer drug delivery systems. Teniposide is a broad spectrum and effective anticancer drug, but its poor water-solubility and adverse effects of commercial formulation (VM-26) restrict its clinical application. In this work, teniposide-loaded polymeric micelles were prepared based on monomethoxy-poly(ethylene glycol)-poly(ε-caprolactone-co-D,L- lactide) (MPEG-PCLA) copolymers through a thin-film hydration method to improve the hydrophilic and reduce the systemic toxicity. The prepared teniposide micelles were without any surfactants or additives and monodisperse with a mean particle size of 29.6 ± 0.3 nm. The drug loading and encapsulation efficiency were 18.53 ± 0.41% and 92.63 ± 2.05%, respectively. The encapsulation of teniposide in MPEG-PCLA micelles showed a slow and sustained release behavior of teniposide *in vitro* and improved the terminal half-life ($t_{1/2}$), the area under the plasma concentration-time curve (AUC) and retention time of teniposide *in vivo* compared with VM-26. In addition, teniposide micelles also enhanced the cellular uptake by MCF-7 breast cancer cells *in vitro* and increased the distribution in tumors *in vivo*. Teniposide micelles showed an excellent safety with a maximum tolerated dose (MTD) of approximately 50 mg teniposide/kg body weight, which was 2.5-fold higher than that of VM-26 (about 20 mg teniposide/kg body weight). Furthermore, the intravenous application of teniposide micelles effectively suppressed the growth of subcutaneous MCF-7 tumor *in vivo* and exhibited a stronger anticancer effect than that of VM-26. These results suggested that we have successfully prepared teniposide-loaded MPEG-PCLA micelles with improved safety, hydrophilic and therapeutic efficiency, which are efficient for teniposide delivery. The prepared teniposide micelles may be promising in breast cancer therapy.

1. Introduction

Cancer is a major public health problem worldwide and is the leading cause of death in the world. Cancer incidence and mortality are increasing year by year. The global burden of cancer is getting heavier and heavier (Siegel et al., 2016). Chemotherapy is a mainly used treatment of cancer and has been proven to be effective in clinics (Elias et al., 2001; Ozols et al., 2006). But due to the poor water solubility of many chemotherapeutic compounds or the serve side effect of the current commercial formulation, their clinical applications were greatly restricted. Thus, some novel delivery strategies are urgently in need to address this issue (Allen and Cullis, 2004).

Teniposide is a semisynthetic derivative of podophyllotoxin with a broad spectrum of *in vivo* antitumor activity (Adiga and Jagetia, 1999; McCowage et al., 1995). The antitumor mechanism of teniposide is related to the inhibition of type II topoisomerase activity and the stabilization of a topoisomerase II–DNA intermediate, thus damaging DNA in replication process and inducing cellular apoptosis (Hartmann and Lipp, 2006; Pommier et al., 1991). Teniposide has been used in the treatment of small cell lung cancer, leukemia, lymphoma, intracranial malignant tumor and other types of cancer (Clark and Slevin, 1987; Feun et al., 2007; Sonneveld, 1992). In addition to this, teniposide is also active against sublines of certain leukemia with acquired resistance to

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cisplatin, doxorubicin, mitoxantrone, or vincristine and so on (Skacel et al., 2013). However, owing to the poor water solubility, the clinical applications of teniposide injection (Vumon®, VM-26) are usually used Cremophor® EL, dehydrated alcohol and benzyl alcohol as excipients, which may cause some side effects, such as hypersensitivity reactions, hypotension, tachycardia or bronchial spasm, and are not well tolerated by some patients (Carstensen et al., 1989; Kubisz et al., 1995; Nolte et al., 1988; Shimizu et al., 1987). In addition, teniposide is free in VM-26, thus leading rapid elimination and widespread tissue distribution including normal organs and tumor tissue, which may decrease the therapeutic efficiency and increase the side effects.

To date, numerous attempts have been made to improve the water solubility and reduce the systemic toxicity of teniposide, such as phospholipid complex albumin nanoparticle, nanosuspensions, PLGA nanoparticles, liposomes and self-assembled nanocarrier (Alkan-Onyuksel and Son, 1992; He et al., 2015a, 2015b; Mo et al., 2012; Zhang et al., 2013). Polymeric micelles are versatile nano-therapeutic platform and have been widely adopted in recent years, which usually self-assemblies of amphiphilic polymers with core-shell nanostructures. The size of polymeric micelle typically ranges from 10 to 100 nm. Polymeric micelles could efficiently encapsulate the hydrophobic drugs into the hydrophobic core to improve the water-solubility of hydrophobic drugs (Chu et al., 2016; Yokoyama, 2010). In addition, amphiphilic polymers usually have good biocompatibility and biodegradability to assure the biosafety in *in vivo* applications. More importantly, the nanoscale drug-loaded micelles could passively target and accumulate in the tumor tissues through the abnormal vasculature via the enhanced permeability and retention (EPR) effect, thus reducing side effects and improving anti-tumor effects (Fang et al., 2011).

Polymeric micelles such as MPEG-PCL have been successfully as the delivery carriers of hydrophobic drug including paclitaxel, docetaxel, curcumin, tacrolimus and others (Gou et al., 2011; Wang et al., 2013a, 2011, 2013b). However, MPEG-PCL micelles delivery systems usually exhibit poor water solubility because of the high crystallinity and hydrophobicity of PCL block, which restricts their further application (Li et al., 2000). In previous work, we attempt to introduce D,L-LA into the PCL block to improve the properties of MPEG-PCL. The prepared monomethoxy-poly(ethylene glycol)-poly(ε-caprolactone-co-D,L-lactide) copolymers (MPEG-PCLA) showed lower crystallinity and higher water solubility compared with MPEG-PCL copolymers. More interestingly, the crystallinity, thermal and hydrolytic properties of MPEG-PCLA can be adjusted by varying the introduction content of D,L-LA, while there is almost no influence of the drug loading capacity.

In this work, we prepared teniposide micelles based on MPEG-PCLA amphiphilic block copolymers with the same content of CL and LA via a thin-film hydration method. Then we investigated the drug loading property, drug release profile, cellular uptake and antitumor effect on breast cancer cell line *in vitro* in detail. Moreover, we also studied the pharmacokinetic behavior, tissue distribution and antitumor activity *in vivo*.

2. Materials and methods

2.1. Materials

Monomethoxy poly(ethylene glycol) (MPEG, Mn = 2000), estradiol valerate and stannous octaoate ($Sn(Oct)_2$) were purchased from Sigma-Aldrich (USA), ε-caprolactone (ε-CL) was purchased from Alfa-Aesar (USA), D,L-lactide (D,L-LA) was bought from Jinan Daigang Biomaterial Co. Ltd (China), Roswell Park Memorial Institute 1640 medium (RPMI 1640, Gibco, USA), Dulbecco's modified Eagle's medium (DMEM, Hyclone, USA). Teniposide and Vumon® were purchased from Dalian Meilun Biology Technology Co., Ltd. (China) and Corden Pharma Latina S.P.A., respectively. Other materials used in this articles were analytical pure and used as received.

Human breast cancer cells (MCF-7) and Human Umbilical Vein Endothelial Cells (HUVEC) were incubated in DMEM supplement with 10% fetal bovine serum (FBS), respectively. The cells were cultured at 37 °C with a humidified 5% CO_2 atmosphere.

Female Sprague-Dawley (SD) rats (200 ± 20 g), BALB/c mice (18 ± 2 g) and BALB/c nude mice (18 ± 2 g) were used for the pharmacokinetic study, maximum tolerated dose study and *in vivo* antitumor tests, respectively. All these animals were purchased from Shanghai Experiment Animal Center (CAS), housed at a controlled environment in the Laboratory Animal Center of Wenzhou Medical University and quarantined for a week before experiment. The experimental procedures were conducted according to Institutional Animal Care and Use guidelines. All the mice were cared humanely throughout the experimental period.

2.2. Synthesis of MPEG-PCLA copolymer

The MPEG-PCLA copolymer with the designed molecular weight of 4000 (2000-1000-1000) was synthesized by ring-opening polymerization of ε-CL and D,L-LA using $MPEG_{2000}$ as an initiator and $Sn(Oct)_2$ as catalyst according to previous reports (Hyun et al., 2006). In brief, MPEG, ε-CL, D,L-LA and $Sn(Oct)_2$ were added into a round-bottom flask, and then reacted at 130 °C for 6 h with mild agitation under a nitrogen atmosphere. The obtained MPEG-PCLA copolymer was purified via the process of precipitation, dialysis and freeze-drying and then stored in air-tight bottles at −20 °C for further use. The ^1H NMR spectra of MPEG-PCLA copolymers were measured by a Varian 400 spectrometer (Varian, Palo Alto, CA).

2.3. Preparation and characterization of teniposide micelles

2.3.1. Preparation of teniposide micelles

The drug-free and teniposide-loaded MPEG-PCLA micelles were prepared by thin-film hydration method. Briefly, the designed amount of MPEG-PCLA copolymers and teniposide with varying ratios were put into a round-bottom flask and then added the mixed solvent of acetonitrile and acetone (1:1, v:v) to well dissolve. Solutions were evaporated via a reduced-pressure in a rotary evaporator at 30 °C and a thin film was formed during this process. The clear solution of teniposide micelles were obtained via hydrating the film in water. Then the solution was filtrated through a 220 nm syringe filter to remove non-entrapped drug. The teniposide micelles were lyophilized to receive dried teniposide micelles powder and stored at 4 °C before use. For drug-free micelles, teniposide was omitted. The preparation scheme of teniposide micelles with specific core-shell structure by self-assembly was shown in Scheme 1.

2.3.2. Characterization of teniposide micelles

2.3.2.1. Drug loading and entrapment efficiency. About 10 mg of lyophilized teniposide micelles were dissolved in acetonitrile/water (45/55, v/v). The concentration of teniposide was determined using reverse-phase high performance liquid chromatography instrument (RP-HPLC, Agilent 1260, USA) with a HC-C18 column (4.6 mm × 150 mm, 5 μm, Agilent, USA) at 227 nm. The column temperature was constant at 30 °C. The mobile phase was acetonitrile/water (45/55, v/v) with a flow rate of 1.0 ml/min. The DL and EE of the teniposide micelles were determined according to the follows: