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The drug encapsulation efficiency, in vitro drug release, cellular uptake and cytotoxicity of paclitaxel-loaded poly(lactide)–tocopheryl polyethylene glycol succinate nanoparticles

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Abstract

Paclitaxel is one of the most effective antineoplastic drugs. Its current clinical administration is formulated in Cremophor EL, which causes serious side effects. Nanoparticle (NP) technology may provide a solution for such poisonous adjuvant problems and promote a sustained chemotherapy, in which biodegradable polymers play a key role. Our group has successfully synthesized novel poly(lactide)–tocopheryl polyethylene glycol succinate (TPGS) (PLA–TPGS) copolymers of desired hydrophobic–hydrophilic balance for NP formulation of anticancer drugs. The present work is focused on effects of the PLA:TPGS composition ratio on drug encapsulation efficiency, in vitro drug release, in vitro cellular uptake and viability of the PLA–TPGS NP formulation of paclitaxel. The PLA–TPGS copolymers of various PLA:TPGS ratios were synthesized by the ring-opening polymerization method and characterized by GPC and ^{1}H NMR for their molecular structure. Paclitaxel-loaded PLA–TPGS NPs were prepared by a modified solvent extraction/evaporation method and characterized by laser light scattering for size and size distribution, scanning electron microscopy for surface morphology and zeta potential for surface charge. High performance liquid chromatography was used to measure the drug encapsulation efficiency and in vitro drug release profile. Cancer cell lines HT-29 and Caco-2 were used to image and measure the cellular uptake of fluorescent PLA–TPGS NPs. Cancer cell viability of the drug-loaded PLA–TPGS was measured by MTT assay. It was found that the PLA:TPGS composition ratio has little effects on the particle size and size distribution. However, the PLA–TPGS NPs of 89:11 PLA:TPGS ratio achieved the best effects on the drug encapsulation efficiency, the cellular uptake and the cancer cell mortality of the drug-loaded PLA–TPGS NPs. This research was also carried out in close comparison with the drug-loaded PLGA NPs.

Keywords: Biodegradable polymers; Cancer; Cancer nanotechnology; Chemotherapy; Taxol®

1. Introduction

Nanoparticle (NP) formulation of anticancer drugs aroused intensive research in the past decades and has become an important area in cancer nanotechnology [1]. NPs of biodegradable polymers can provide a way of sustained, controlled and targeted drug delivery to improve the therapeutic effects and reduce the side effects of the formulated drugs [2]. Poly(lactide) (PLA), poly(D,L-lactide-co-glycolide) (PLGA), and poly(caprolactone) (PCL) are FDA-approved biodegradable polymers, which are used most often in the literature of drug delivery [3–5]. These polymers were originally synthesized to make surgical sutures and thus have disadvantages to be used for drug formulation, including too high hydrophobicity (thus not friendly to hydrophilic drugs) and too slow degradation (thus too slow drug release). Novel biodegradable polymers/copolymers with desired hydrophobic/hydrophilic balance and desired degradation rate are thus needed.

In the literature, PLGA NPs were usually prepared by using chemical emulsifiers such as poly(vinyl alcohol)

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(PVA), which has been found of disadvantages including low emulsification efficiency, side effects and difficulties to wash away in the formulation process. Instead, D-α-tocopheryl polyethylene glycol 1000 succinate (vitamin E TPGS or simply, TPGS) has high emulsification efficiency (67 times higher than PVA). It can also greatly improve the drug encapsulation efficiency (up to 100% EE achieved) and enhance cellular uptake of NPs and thus increase the cancer cell mortality [6–9]. TPGS is a water-soluble derivative of natural vitamin E and its hydrophile–lipophile balance (HLB) which is used to express the surfactant molecular hydrophilic or lipophilic strength is ~13. When surfactants of HLB greater than 10 show hydrophilic property while those of less than 10 are more lipophilic [10]. The chemical structure of TPGS is similar to other amphiphiles comprising lipophilic alkyl tail and hydrophilic polar head portion. Its bulky structure and large surface area characteristics make it an excellent emulsifier. Moreover, it has been found that co-administration of TPGS could enhance cytotoxicity, inhibit *P*-glycoprotein-mediated multi-drug resistance, and increase the oral bioavailability of anticancer drugs [11–16]. This triggered us to synthesize PLA–TPGS copolymers to take such advantages of TPGS for NP formulation of anticancer drugs, which can be expected to have self-emulsification effects (no emulsifiers are needed for the NPs formulation) and achieve appropriate drug encapsulation efficiency up to 62% and desired drug release profiles. We showed in our earlier work that PLA–TPGS NPs can be prepared with or without TPGS as surfactant. Also, we found that with 0.03% TPGS used as emulsifier high drug encapsulation efficiency of up to 89% can be achieved. Therefore we used 0.03% TPGS as emulsifier in this work [17].

In this work we extended our earlier research on PLA–TPGS copolymer synthesis for NP formulation of anticancer drugs with emphasis on the effects of PLA:TPGS composition ratio on physicochemical properties, in vitro drug release, in vitro cellular uptake and cytotoxicity of paclitaxel-loaded PLA–TPGS NPs. The work is also carried out in comparison with the PLGA NPs. The molecular structure of the synthesized copolymers of various PLA:TPGS composition ratios were first characterized by ^{1}H NMR and GPC. Paclitaxel-loaded PLA–TPGS NPs of various PLA:TPGS composition ratio were fabricated by a modified solvent extraction/evaporation technique, which were then characterized by various state-of-the-art techniques such as laser light scattering (LLS) for size and size distribution, field emission scanning electron microscopy (FESEM) for surface morphology and zeta-potential for surface charge. High-performance liquid chromatography (HPLC) was used to measure the drug encapsulation efficiency (EE) and the in vitro drug release kinetics. Caco-2 and HT-29 cells were used for in vitro cell line experiment. Cellular uptake of fluorescent PLA–TPGS NPs was imaged by confocal laser scanning microscopy (CLSM) and quantitatively measured by microplate reader. Cytotoxicity of paclitaxel-loaded PLA–TPGS NPs were investigated by MTT assay and IC_{50} was analyzed.

2. Materials and methods

2.1. Materials

Lactide (3, 6–dimethyl-1, 4–dioxane-2, 5-dione, $C_6H_8O_4$) was purchased from Aldrich and recrystallized twice in ethyl acetate. TPGS (NF grade) was obtained from Eastman Chemical Company and freeze-dried for 2 days before polymerization. Stannous octoate was purchased from Sigma and used with 1% distillated toluene formulation. Paclitaxel was purchased from Dabur India Limited, India. Poly (DL-lactide-co-glycolide) (PLGA, 50:50, Mw: 40,000–75,000), polyvinyl alcohol (PVA, Mw: 30,000–70,000) and MTT ([3-4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) were also purchased from Sigma. All other chemicals including ethyl acetate from Merck; DCM (dichloromethane) and acetonitrile from Aldrich; and methanol from Fisher Chemicals were of HPLC grade and used without further pre-treatment. All reagent water used in the laboratory was pretreated with the Milli-Q Plus System (Millipore Corporation, Bedford, USA).

2.2. Synthesis and characterization of PLA–TPGS copolymers

PLA–TPGS copolymers of various PLA:TPGS composition ratio were synthesized by ring-opening polymerization mechanism with the presence of lactide and TPGS [18]. Preweighed portions of lactide and TPGS were added into a glass ampoule together with 0.5 wt% stannous octoate catalyst solution. The ampoule was then immersed in liquid nitrogen and evacuated for 45 min to remove moisture and oxygen from the reagents. The ampoule was then sealed with a butane burner and allowed to react at 145 °C in silicone oil bath for 12 h. At the end of the polymerization, the product was dissolved in DCM and precipitated in cold methanol to remove the unreacted monomers. The precipitated copolymer was subsequently filtered and vacuum-dried for 2 days at 45 °C. Nuclear magnetic resonance (300 MHz ^{1}H NMR, Bruker ACF 300) spectroscopy was used in the determination of number-average molecular weight of the copolymer. The molecular weight and weight distribution of the PLA-TPGS copolymers of various PLA:TPGS composition ratios was determined by gel permeation chromatography (GPC, Agilent 1100 series GPC analysis system with RI-G1362A refractive index detector). The molecular structure of PLA–TPGS copolymer is shown in Fig. 1.

2.3. Formulation of paclitaxel-loaded PLA–TPGS NPs

The paclitaxel-loaded PLA–TPGS NPs were fabricated by a modified solvent extraction/evaporation method [7, 17]. In brief, given amount of paclitaxel and 50 mg PLA–TPGS copolymer were dissolved in 4 ml DCM and vortexed for 60 s. The formed solution was poured into 60 ml 0.03% (w/v) TPGS water solution under gentle stirring. The mixture was sonicated for 120 s at 25 W output. The emulsion was evaporated overnight under magnetic stirring to remove organic solvent. The particle suspension was filtered through 1.2 μm membrane filter and then centrifuged at 11,500 rpm for 15 min. The pellet was then resuspended at 10 ml water and freeze-dried for2 days to get the NPs powder.

2.4. Characterization of NPs

2.4.1. Particle size and size distribution

Average size and size distribution of the drug-loaded NPs were measured by the laser light scattering technique (Brookhaven Instruments Corporation 90-PLUS analyzer). Samples for 90-plus measurement were prepared by diluting the NPs suspension with deionized water and sonicated for 30 s before measurement to ensure that the particles were well dispersed and the dispersion was homogeneous.