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Vitamin E TPGS prodrug micelles for hydrophilic drug delivery with neuroprotective effects

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ABSTRACT

Double emulsion has been used most often in formulation of hydrophilic drugs by nanoparticles of biodegradable polymers, which has disadvantages such as low drug loading and low drug encapsulation efficiency due to the drug loss in the process. The drug release may be too fast for sustained chemotherapy. We developed in this research a D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) prodrug micelle system with cisplatin as a model hydrophilic drug. We demonstrated that such a system can successfully deliver the model hydrophilic drug with a low critical micelle concentration (CMC) of only 5.01 mg/L, a high drug load of 4.95% (w/w) and a pH-responsive drug release kinetics and higher cellular uptake in comparison with the original drug and the TPGS-cisplatin prodrug itself. The cell viability experiment showed great enhancement of the cisplatin chemotherapy, which is demonstrated by the IC50 value reduced from 3.95, 0.98, 0.19 for cisplatin to 1.36, 0.51, 0.08 μg/mL for the TPGS prodrug micelle formulation after 24, 48, 72 h culture with the HepG2 hepatocarcinoma cells, respectively. Furthermore, such a TPGS prodrug micellar formulation showed significant neuroprotective effects for the cisplatin chemotherapy, which is demonstrated by the greatly increased IC50 value for the SH-SY5Y neuroblast-like cells in comparison between cisplatin and the TPGS prodrug micelle formulation. The TPGS prodrug micelles can also be generalized to become a new strategy for codelivery of hydrophilic and hydrophobic drugs and/or imaging agents.

1. Introduction

Nanoparticles of biodegradable polymers have been found promising for formulation of hydrophobic anti-cancer drugs such as paclitaxel and docetaxel with high loading and high drug encapsulation efficiency as well as high cellular uptake (Chan et al., 2010; Cho et al., 2008; Davis et al., 2008; Feng, 2011; Feng and Chien, 2003; Feng et al., 2007; Peer et al., 2007; Shi et al., 2010). Single emulsion and double emulsion techniques are applied to synthesize polymeric nanoparticles for formulation of hydrophobic and hydrophilic drugs, respectively. The single emulsion technique is inappropriate to be applied to deliver hydrophilic drugs due to the hydrophobic nature of the nanoparticle core. Instead, the double emulsion technique should be applied to synthesize nanoparticles, in which the hydrophilic drug is dissolved in a water phase that is added in an oil phase to form the water-in-oil phase. Such a first phase is then added to a water phase to form the water-in-oil-water phase. As such, the double emulsion technique has disadvantages for hydrophilic drug delivery, which include (1) low drug loading (*i.e.*, the weight ratio of the drug to the nanoparticle) and low drug encapsulation efficiency (the encapsulated drug amount *versus* the total drug amount added in the process) due to the drug loss into the water phase in the process, (2) the large initial burst (the fast drug release in the first hour), (3) undesired drug release due to the fast diffusion of the drug within the nanoparticles and (4) the undesired particle size (more than 200 nm) due to the complicated emulsion process (Lee et al., 2007).

Prodrug is a strategy used most often in drug formulation, in which the drug is conjugated to a polymer. The prodrug carries the drug to the site and then releases the drug for action there. It improves the pharmacokinetics of the original drugs, increasing the half-life of the drugs in blood (Cao and Feng, 2008). The disadvantages of prodrugs, however, include low drug loading and low cellular uptake of the prodrugs due to their intrinsic structure. It is thus natural to synthesize prodrug nanohydrogels, prodrug micelles, prodrug liposomes, prodrug loaded nanoparticles of biodegradable polymers to make use of their advantages and avoid their disadvantages, among which prodrug micelles have

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received high attention recently due to their technical simplicity and promising performance in *in vitro* experiments. For example, this research will be focused on conjugation of hydrophilic drug to an amphiphilic biodegradable copolymer and use of the prodrug to form micelles.

In this research, cisplatin (cis-diamminedichloroplatinum II) is selected as a model hydrophilic drug to demonstrate the concept of prodrug micelles for hydrophilic drug formulation. Cisplatin shows anti-cancer effect for a variety of cancers including breast, head, neck, testicular, ovarian, bladder and small cell lung cancers (Ho et al., 2003; Jamieson and Lippard, 1999; Kelland, 2007; Rosenber et al., 1969). The mechanism of the drug is to form platinum–DNA adducts after being uptaken into the nucleus of cells and prevent the strands from uncoiling and separating (Wang and Lippard, 2005). However, cisplatin shows limitations in clinical application. The main limitation is its low solubility and high toxicity. Cisplatin is difficult to be well dispersed in the plasma because it is poorly soluble in organic solvents and partially soluble in water (Fujiyama et al., 2003). Besides, patients will suffer from serious side effects including neurotoxicity, emetogenesis and nephrotoxicity. Because nephrotoxicity and emetogenesis can be significantly reduced by hyperhydration, neurotoxicity is a main dose-limiting toxic effect of cisplatin (Decatris et al., 2004; Wong and Giandomenico, 1999). It will lead to peripheral neuropathy, tinnitus and high-frequency hearing loss.

Many kinds of molecular biomaterials have been used to form drug delivery systems, which include US FDA approved biodegradable polymers such as poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), poly(ε-caprolactone) (PCL), are used most often (Barreto et al., 2011). However, such polymers could not achieve desired effects in prodrug formulation since they were originally synthesized for textile grafts and implants in the 1950s. They are highly hydrophobic and thus not friendly to hydrophilic drugs. They have too strong mechanical strength and their degradation is too slow, thus resulting in too slow drug release to meet the therapeutic needs. Also, the prodrugs made up of those polymers are difficult to be directly conjugated to hydrophilic molecular probes for targeting (Feng, 2006). Synthesized biodegradable polymers are thus preferred. For example, Zhang's group synthesized poly(ethylene glycol)-*b*-poly(L-lactide) (PEG-PLA) and conjugated it to cisplatin to form a prodrug, which was composed of a cisplatin complex with two ketobearing ligands conjugated to two PEG-PLA chains *via* an acid responsive Schiff-base linkage. The prodrug displayed abilities to kill A2780 human ovarian carcinoma cells several times more than free cisplatin. Nevertheless, the drug loading is only 1 wt% (Aryal et al., 2010; Hans et al., 2005). Jing's group synthesized a biodegradable amphiphilic tri-block copolymer, MPEG-*b*-PCL-*b*-PLL, which contains pendant amino groups to form a polymeric prodrug of cisplatin. The prodrug was then used to form micelles. The resulted micelles were quite large with an average diameter of about 200–220 nm without cisplatin and 150–160 nm with cisplatin. Also, the therapeutic effects of such prodrug micelles were not enhanced significantly (Xiao et al., 2011).

We propose to synthesize D-α-tocopheryl polyethylene glycol 1000 succinate (Vitamin E TPGS or simply TPGS) prodrug micelles as a platform to formulate hydrophilic drugs to achieve high drug loading, high drug encapsulation efficiency and high cellular uptake of the formulated drug. Vitamin E TPGS or simply TPGS (D-alpha-tocopheryl polyethylene glycol 1000 succinate) is a water-soluble derivative of natural vitamin E, *i.e.*, a PEGylated Vitamin E, which has amphiphilic structure comprising lipophilic alkyl tail and hydrophilic polar head portion. TPGS already showed advantages in formulation of various anti-cancer drugs such as doxorubicin, which showed enhanced therapeutic effects and reduced side effects (Anbharasi et al., 2010; Cao and Feng, 2008). Cis, trans, cis-$Pt(NH_3)_2Cl_2(OH)_2$ is an analog of cisplatin. This Pt (IV) analog molecule can be further esterified with carboxyl or acid anhydride groups (Aryal et al., 2010; Lippard et al., 2008, 2011; Xiao et al., 2011). Therefore, through a linker with carboxyl group, it is possible to conjugate the drug with TPGS to form a prodrug. This prodrug can further be used to form micelles in water to enhance drug loading and cellular uptake. Such a formulation of prodrug micelles solves the problem of poor solubility of cisplatin and prolongs its half-life in circulation in comparison with original cisplatin. Besides, the ester bond would be stable in the blood (pH = 7.4) but easily cleaved in endosomes (pH = 5–6) after uptake by cancer cells (Barnes et al., 2004). The anti-cancer effect of such prodrug micelles can be shown *in vitro* with a cancer cell line HepG2. When compared with direct encapsulation of cisplatin, such prodrug micelles are able to load other therapeutic or imaging agents into the core of micelles to achieve combination therapy or theranostics. For example, many literatures have reported that cisplatin and docetaxel can be combined to achieve synergistic efficacy (Chang et al., 2011; Kodama et al., 2009; Ridwelski et al., 2001).

Moreover, since vitamin E TPGS has shown certain neuroprotective effects of the cisplatin chemotherapy, such Vitamin E TPGS-cisplatin prodrug micelles can further reduce the side effects for cisplatin treatment (Naziroglu et al., 2004; Pace et al., 2003). It is reported that such neuroprotective effects of TPGS for cisplatin treatment is currently under phase III clinical trial (Argyriou et al., 2005, 2006; Pace et al., 2010). The results showed that Vitamin E supplementation should be adopted in patients receiving cisplatin-based chemotherapy to reduce the neurotoxicity. We shall demonstrate the neuroprotective effect of such prodrug micelles *in vitro* on a human neuroblastoma cell line SH-SY5Y which is a common model for neurotoxicity test (Harry et al., 1998; Hu et al., 2012; Nicolini et al., 1998).

2. Materials and methods

2.1. Materials

Vitamin E TPGS (D-α-tocopheryl polyethylene glycol 1000 succinate, $C_{33}O_5H_{54}(CH_2CH_2O)_{23}$) was from Eastman Chemical Company (Tennessee, USA). Cis-diammineplatinum (II) dichloride, succinic anhydride, N,N′-dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), dimethyl sulfoxide (DMSO), coumarin-6, phosphate buffered saline (PBS, pH 7.4), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, trypsin-EDTA solution and propidium iodide (PI), poly(ethylene glycol) (PEG, M_n = 1000) were all purchased from Sigma–Aldrich (St. Louis, MO, USA). Tween-80 was from ICN Biomedicals, Inc. (OH, USA). Triton X-100 was provided by USB Corporation (OH, USA). Fetal bovine serum (FBS) was purchased from Gibco Life Technologies (AG, Switzerland). Penicillin–streptomycin solution was from Invitrogen (CA, USA). Dulbecco's Modified Eagle's Medium (DMEM) was from Sigma (St. Louis, MO, USA). All solvents used in this study were HPLC grade. HepG2 cancer cells and SH-SY5Y cells were provided by American Type Culture Collection (ATCC, USA). The water used was pretreated with the Milli-Q® Plus System (Millipore Corporation, Bedford, USA).

2.2. Synthesis of TPGS-cisplatin and cisplatin-PEG prodrugs

For synthesis of TPGS-cisplatin prodrug, cis, cis, trans-diamminedichlorodihydroxyplatinum and cis, cis, trans-diamminedichlorodisuccinatoplatinum (IV) (cisplatin-SA) were first synthesized according to previous methods (Barnes et al., 2004; Ellis et al., 1995). TPGS, cis, cis, trans-diamminedichlorodisuccinatoplatinum (IV), DCC and DMAP with a stoichiometric molar ratio of 1:10:10:0.1 were then dissolved in DMSO and stirred under