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Cholesterol-modified poly(lactide-co-glycolide) nanoparticles for tumor-targeted drug delivery

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ABSTRACT

Poly(lactide-co-glycolide)-cholesterol (PLGA-C)-based nanoparticles (NPs) were developed for the tumor-targeted delivery of curcumin (CUR). PLGA-C/CUR NPs with ~200 nm mean diameter, narrow size distribution, and neutral zeta potential were fabricated by a modified emulsification-solvent evaporation method. The existence of cholesterol moiety in PLGA-C copolymer was confirmed by proton nuclear magnetic resonance (^{1}H NMR) analysis. *In vitro* stability of developed NPs after 24 h incubation was confirmed in phosphate buffered saline (PBS) and serum media. Sustained (~6 days) and pH-responsive drug release profiles from PLGA-C NPs were presented. Blank PLGA and PLGA-C NPs exhibited a negligible cytotoxicity in Hep-2 (human laryngeal carcinoma) cells in the tested concentration range. According to the results of flow cytometry and confocal laser scanning microscopy (CLSM) studies, PLGA-C NPs presented an improved cellular accumulation efficiency, compared to PLGA NPs, in Hep-2 cells. Enhanced *in vivo* tumor targetability of PLGA-C NPs, compared to PLGA NPs, in Hep-2 tumor-xenografted mouse model was also verified by a real-time near-infrared fluorescence (NIRF) imaging study. Developed PLGA-C NPs may be a candidate of efficient and biocompatible nanosystems for tumor-targeted drug delivery and cancer imaging.

1. Introduction

Many approaches have been tried to deliver anticancer agents to tumor site selectively *via* intravenous route recently (Cho et al., 2012; Lee et al., 2015; Park et al., 2014; Ryu et al., 2012; Shu et al., 2014). Maximized drug delivery to tumor region and minimized distribution to normal tissues and organs can be accomplished by tumor targeting strategies. Due to the anatomical-physiological characteristics of solid tumors, such as defective vascular architectures and immature lymphatic drainage systems, an enhanced vascular permeability can be shown. Due to the enhanced permeability and retention (EPR) effect, macromolecules can be extravasated from tumor blood vessels and they can be accumulated in the tumor tissues (Matsumura and Maeda, 1986; Torchilin, 2011). The EPR effect can be used as a passive tumor targeting strategy for chemotherapy of cancers *via* intravenous route. Various nanocarriers, with specific physicochemical characteristics (*i.e.* size, surface charge, and shape), have been developed using an EPR effect as a tumor targeting strategy (Bertrand et al., 2014). However, because of its intrinsic lack of tumor specificity, an active tumor targeting based on ligand and receptor interaction has been introduced (Basile et al., 2012; Mohanty et al., 2011). Various ligands (*i.e.* small chemicals, proteins, and peptides) have been used to deliver nanocarriers exactly to receptors expressed on cancer tissues (Zhong et al., 2014). By combining passive and active tumor targeting strategies, the accuracy of tumor targetability can be improved.

To fabricate nanosystems for the delivery of anticancer agents to tumor region, diverse materials, including natural and synthetic polymers, have been used (Merkle, 2015; Sun et al., 2015). Among them, poly(lactide-co-glycolide) (PLGA) has been regarded as one of biocompatible and biodegradable materials (Choi et al., 2012; Danhier et al., 2012). Besides PLGA itself, a number of PLGA derivatives have been investigated to prepare nano-sized drug delivery systems (Dhar et al., 2008; Yoon et al., 2015). In this study, poly(lactide-co-glycolide)-cholesterol (PLGA-C) has been introduced to fabricate nanoparticles (NPs) for tumor-targeted drug delivery and cancer imaging for the first time. Cholesterol is one of endogenous substances involved in many biological functions. Given the roles of cholesterol in the endocytosis of materials and cancer proliferation and metastasis (Iversen et al., 2011; Khalil

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et al., 2006), it is expected that PLGA-C NPs have an augmented tumor-targetability compared to PLGA NPs.

CUR is a linear diarylheptanoid and it is one of curcuminoids of turmeric. It is isolated from *Curcuma longa* and can be existed as a few tautomeric forms, including keto and enol forms (Naksuriya et al., 2014). CUR has a lot of pharmacological efficacies, such as anticancer, anti-inflammatory, antimicrobial, antioxidant, anti-rheumatic, hypoglycemic, and nephron-protective effects (Agrawal and Mishra, 2010; Anand et al., 2007; Dulbecco and Savarino, 2013; He et al., 2015; Meng et al., 2013). Especially, its anticancer activities, including antiangiogenic, antiapoptotic, anti-inflammatory, antineoplastic, chemopreventive, and chemosensitizing effects, have been verified in several cancer cells (Bar-Sela et al., 2010). In spite of its various anticancer activities, its poor physicochemical properties, such as poor aqueous solubility, degradability in the alkaline condition, and photodegradability, may restrict its clinical use (Naksuriya et al., 2014; Priyadarsini, 2009; Tønnesen et al., 2002). As well as oral formulations of CUR, many injection formulations of CUR for chemotherapy have been developed (Song et al., 2014; Yang et al., 2015; Yoon et al., 2015).

Herein, we developed PLGA-C NPs for the delivery of CUR to tumor region *via* intravenous route. Physicochemical properties, *in vitro* stability, *in vitro* drug release, *in vitro* cellular uptake, and *in vivo* tumor targetability of developed PLGA-C NPs were investigated.

2. Materials and methods

2.1. Materials

PLGA (average Mn: 40 kDa) and PLGA-C (average Mn: 38 kDa) were purchased from PolySciTech (Akina, Inc., West Lafayette, IN, USA). CUR, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI), and poly(vinyl alcohol) (PVA) were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), penicillin, streptomycin, phosphate buffered saline (PBS), and fetal bovine serum (FBS) were purchased from Gibco Life Technologies, Inc. (Carlsbad, CA, USA). All other reagents were of analytical grade and were obtained from commercial sources.

2.2. Preparation and characterization of CUR-loaded NPs

The characteristic of PLGA-C was investigated by proton nuclear magnetic resonance (^{1}H NMR) spectroscopic method. For ^{1}H NMR (500 MHz) analysis, PLGA-C was solubilized in deuterated chloroform ($CDCl_3$).

CUR-loaded PLGA and PLGA-C NPs were fabricated using a modified emulsification-solvent evaporation method (Lee et al., 2014; Yoon et al., 2015). In brief, PLGA or PLGA-C (40 mg) and CUR (4 mg) were solubilized in dichloromethane (1 mL) and that polymer/drug solution was added to 8 mL PVA solution (2%, w/v). That emulsion was sonicated for 20 min using a probe sonicator (VC-750; Sonics & Materials, Inc., Newtown, CT, USA). By stirring for 30 min at room temperature, the organic solvent was removed. The aqueous suspension of NPs was then centrifuged at 15,000 rpm for 40 min and the supernatant was eliminated. The pellets of NPs were resuspended in 8 mL distilled deionized water (DDW). The centrifugation and resuspension processes were repeated three times to remove unencapsulated drug and PVA completely.

CUR-loaded NPs were freeze-dried after adding 3% (w/v) sucrose (as a cryoprotectant) and stored for further uses.

The particle size, polydispersity, and zeta potential of fabricated NPs were measured by dynamic light scattering (DLS) and laser Doppler methods (ELS-Z1000; Otsuka Electronics, Tokyo, Japan). The encapsulation efficiency (EE) of CUR in NPs was quantitatively determined using a high- performance liquid chromatography (HPLC) assay as reported (Yoon et al., 2015). NPs were dissolved in dimethyl sulfoxide (DMSO) and diluted with mobile phase of HPLC assay. Drug content was quantitatively determined using a HPLC system equipped with a pump (PU-2089 Plus; Jasco, Tokyo, Japan), an automatic injector (AS-2050 Plus), and an UV/Vis detector (UV-1575). The mobile phase consisted of acetonitrile, tetrahydrofuran, and DDW (35:20:45, volume ratio) and a reverse phase C18 column (Gemini, 250 mm × 4.6 mm, 5 μm; Phenomenex, Torrance, CA, USA) with guard column (SecurityGuard Guard Cartridge kit, Phenomenex, Torrance, CA, USA) were used. The flow rate was maintained at 1 mL/min and the injection volume was 20 μL. The eluent was monitored at 425 nm by UV/Vis detector. The inter- and intra-day variances were within the acceptable range. The morphology of developed NPs was observed using transmission electron microscopy (TEM). A suspension of drug-loaded NPs was stained with 2% (w/v) phosphotungstic acid, placed on the copper grids with films, dried for 10 min, and observed by TEM (JEM 1010; JEOL, Tokyo, Japan).

2.3. In vitro *stability of NPs*

The stability of PLGA/CUR NPs and PLGA-C/CUR NPs in PBS (pH 7.4) and 50% (v/v) serum (FBS) was assessed by measuring their mean diameters. Freeze-dried NPs were dispersed in each medium (at 5 mg/mL) and the mean diameter of NPs was measured using a DLS method as described in above section. The mean diameters of PLGA/CUR NPs and PLGA-C/CUR NPs were measured at 0 h (pre) and 24 h.

2.4. In vitro *drug release*

The release pattern of CUR from fabricated NPs was evaluated in PBS media (pH 5.5, 6.8, and 7.4). Each CUR-loaded NP dispersion (150 μL) was loaded into a mini GeBA-flex dialysis tube (14 kDa molecular weight cut-off; Gene Bio-Application Ltd., Kfar Hanagide, Israel). That dialysis tube was then immersed in 10 mL PBS (pH 5.5, 6.8, and 7.4 adjusted with phosphoric acid) including 0.1% (w/v) Tween 80. Those samples were then incubated in a shaking

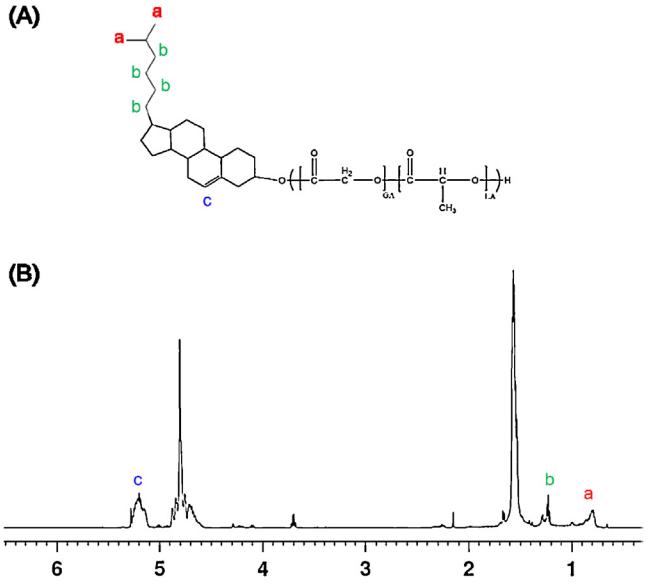

Fig. 1. Properties of PLGA-C copolymer. (a) Chemical structure of PLGA-C is presented. (b) ^{1}H NMR (500 MHz) spectrum of PLGA-C dissolved in $CDCl_3$ is shown.