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Core–shell structured gel-nanocarriers for sustained drug release and enhanced antitumor effect

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Oleoyl macrogol-6 glycerides (PubChem CID: 6435863)
Polysorbate 80 (PubChem CID: 5281955)
Trehalose (PubChem CID: 7427)
Trypsin (PubChem CID: 72699210)
Calcium dichloride (PubChem CID: 5284359)
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A B S T R A C T

The present paper attempted to develop temperature-sensitive and core–shell structured gel-nanocarriers (gel-NCs) for paclitaxel (PTX) with 12-hydroxystearic acid (12-HSA) as an organic gelator, which aims at sustaining drug release over time and thus improves the therapeutic effect. The gel-NCs were prepared by a mechanical mixing and high-pressure homogenization method. The gelation transition temperature (T_{gel}) of the organic phase contained in the cores of the gel-NCs was optimized by a stirring method. The gel-NCs were characterized in terms of the particle size, morphology and in vitro drug release. The in vivo studies, including the antitumor effects on H22 tumor-bearing mice, biocompatibility and toxicity in mice, were performed. Gel-NCs with approximately 170 nm were prepared successfully, and the gelation of the liquid cores at 37 °C was achieved, while the amount of gelator was 3.75% (w/w). Due to the gelation of the cores, sustained drug release over time was obtained. Moreover, the PTX-loaded gel-NCs suppressed tumor growth more efficiently than the conventional nanocarriers with better in vivo biocompatibility and no toxicity to other healthy organs. In conclusion, the 12-HSA organogel-based NCs appear to be promising systems for the sustained release of active compounds for a long time and thus improve the therapeutic outcome.

1. Introduction

Nanoscale drug delivery systems (NDDSs) are one of the most promising approaches to revolutionizing disease diagnosis and therapy, with more than 20 nanoformulations, such as Doxil®, DaunoXome® and Abraxane®, being approved by FDA for clinical use in the past 30 years (Jain and Stylianopoulos, 2010; Koudelka and Turanek, 2012; Lee et al., 2010). The application of NDDSs to the therapy of many diseases could provide therapeutic effects that cannot be achieved with free drugs, owing to advantages including improved drug solubility, enhanced drug stability, prolonged half-life, enhanced absorption of the drugs into a target tissue, improved cellular internalization and organelle-specific delivery, a change in the pharmacokinetic and drug tissue distribution profile, decreased drug resistance, and reduced side effects (Biswas and Torchilin, 2014; Peer et al., 2007; Tao et al., 2013b). The current NDDSs includes liposomes, nanoemulsions, polymersomes, core–shell nanocarriers, polymeric micelles and other options (Lee et al., 2011; Petros and DeSimone, 2010; Sun et al., 2012). Of the NDDSs, core–shell structured nanocarriers based on the lipid-cores have unique advantages over other NDDSs because of their high drug-loading capacity, which can increase the drug bioavailability at action sites and decrease the premature drug release that could help to improve the therapeutic effects (Couvreur et al., 2002; He et al., 2013; Nassar et al., 2009; Shen et al., 2010).

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In previous reports (He et al., 2013, 2014), we developed a novel and simple method for the preparation of core–shell structured nanocarriers (NCs) based on nanoemulsion-templates stabilized by beta-lactoglobulin (β-LG), in which the nanoemulsion-template generation and shell crosslinking were performed simultaneously. Moreover, no unfavorable materials such as surfactants and organic solvents were involved in the preparation of the nanocarriers, which indicates better biocompatibility. Importantly, such a nanocarrier system had perfect stability and drug-loading capacity for poorly water-soluble drugs.

Paclitaxel (PTX), a highly hydrophobic drug, is one of the most often used anti-cancer drugs in clinics for the treatment of various tumors, such as ovarian cancer, non-small cell lung cancer and breast cancer, and it works by first inhibiting the microtubule dynamic instability, which is required for cellular division, and then provoking cell apoptosis (Giuffrida et al., 2014; Schiff et al., 1979). However, its clinical use is hampered by its poor water-solubility, non-specific distribution throughout the body (which causes insufficient penetration into tumors), toxicity to healthy tissues (which limits the dose and frequency of the treatment), and cancer cell resistance (Koudelka and Turanek, 2012; Zhang et al., 2013b).

On the other hand, to obtain a better therapeutic effect, patient compliance and safety and to reduce the administration times, sustained drug release over time is in general warranted. However, sustained release from the conventional NDDSs is very challenging because of the inefficient drug loading and pronounced burst release (Natarajan et al., 2014; Vasir and Labhasetwar, 2007).

An organogel that is composed of organic liquid and organic gelator is a semi-solid system (Hsueh et al., 2010; Skilling et al., 2014; Vintiloiu and Leroux, 2008); it is prepared by warming a gelator in an organic liquid until the solid dissolves and then cooling the solution to below the gelation transition temperature (T_{gel}) (under which the liquid is immobilized over long periods) (George and Weiss, 2006; Hsueh et al., 2010; Terech and Weiss, 1997). 12-hydroxystearic acid (12-HSA), a low-molecular-weight gelator, can self-assemble into thermoreversible molecular networks and form organogels through intermolecular forces such as hydrogen bonding and π interactions in various organic solvents (Chen et al., 2008; Terech et al., 2000). Interestingly, a hot organic solution that contains 12-HSA would change into solid gel once the ambient temperature declines to the T_{gel}. It is therefore hypothesized that by introducing the organic gelator, 12-HSA, into the organic phase used in the preparation of our previous NCs at a fixed ratio (He et al., 2013), the nanoscale liquid cores of the NCs would in theory become "solid nanogel" at body temperature when they are injected into the body, thus achieving sustained drug release. Thus, in this study, we develop temperature-sensitive gel-NCs with a core–shell structure based on 12-HSA for the purpose of sustaining PTX release over time and thus improving the therapeutic effect. To obtain a proof of concept, various studies have been performed, including the characterization of gel-NCs, in vitro drug release, antitumor effects and biocompatibility.

2. Materials and Methods

2.1. Materials

12-HSA (more than 75% purity) was obtained from Tokyo KaSei Industry Co., Ltd. (Tokyo, Japan). The PTX with more than 99% purity was obtained from Yunnan Hande Bio-Tech Co., Ltd. (Kunming, China). The Taxol was obtained from Bristol-Myers Squibb (China) Investment Co., Ltd. (Shanghai, China). The β-LG (90% purity) and coumarin-6 (C-6) were from Sigma–Aldrich Co. Ltd. (St. Louis, MO, USA). The Labrafil M1944CS that was used as an organic phase to dissolve PTX was a gift from Gattefossé Co. (Saint Priest, Cedex, France). H22 cells were purchased from Nanjin KeyGEN Biotech Co., Ltd. (Nanjing, China). The fetal bovine serum, HBS, RPMI-1640, Dulbecco's modified Eagle medium and trypsin were obtained from Thermo Fisher Scientific Inc. (Waltham, MA, USA). The HE Staining Kit was purchased from Beyotime Institute of Biotechnology (Haimen, China). The CD68 antibody was from Wuhan Boster Biological Technology Co., Ltd. (Wuhan, China). All of the other chemicals were of analytical reagent grades and were obtained from Sinopharm Chemical Reagent (Shanghai, China).

Male ICR mice (18–22 g) were purchased from College of Veterinary Medicine Yangzhou University (license no: SCXK (Su) 2012-0004, Yangzhou, China). The animals used in the experiments received care in compliance with the Principles of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals. The experiments followed the protocol approved by the China Pharmaceutical University Institutional Animal Care and Use Committee.

2.2. Preparation of gel-NCs

The gel-NCs were prepared using a method similar to our previous report (He et al., 2013). Briefly, the organic phase was prepared by the procedure that PTX (45 mg) and 12-HSA (60 mg) were dissolved in 2 mL LABRAFIL M1944CS at 80 °C using an ultrasonic dispersion method, and then, 0.08 mL 4 M $CaCl_2$ was dispersed into the LABRAFIL M1944CS by vortex mixing for 10 min. Subsequently, the organic phase was added to 30 mL aqueous solution that contained 1% β-LG (w/w, pH 8.5), which was denatured at 85 °C for 30 min before use. Finally, the mixture was dispersed at 10,000 rpm using a high-speed disperser (Ningbo Scientz Biotechnology Co. Ltd., China) and homogenized at 500 bars for 20 cycles using an AH-2010 high pressure homogenizer (ATS Engineering Inc., Canada). PTX-loaded NCs without gelator and C-6-loaded gel-NCs were prepared with the same procedure except that PTX was dissolved in 2 mL of LABRAFIL M1944CS without 12-HSA and that 6 mg of C-6 was dissolved in 2 mL of LABRAFIL M1944CS that contained 12-HSA in advance, respectively.

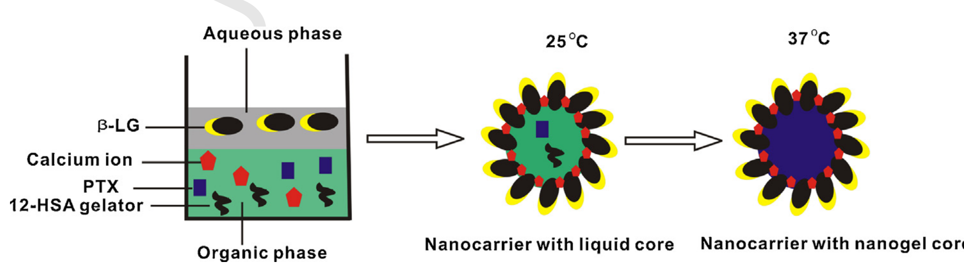

Fig. 1. Schematic illustration of the preparation of the gel-NCs and their structure.

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