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Genistein-loaded nanoparticles of star-shaped diblock copolymer mannitol-core PLGA–TPGS for the treatment of liver cancer

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ABSTRACT

The purpose of this research is to develop nanoparticles (NPs) of star-shaped copolymer mannitol-functionalized PLGA–TPGS for Genistein delivery for liver cancer treatment, and evaluate their therapeutic effects in liver cancer cell line and hepatoma-tumor-bearing nude mice in comparison with the linear PLGA nanoparticles and PLGA–TPGS nanoparticles. The Genistein-loaded M-PLGA–TPGS nanoparticles (MPTN), prepared by a modified nanoprecipitation method, were observed by FESEM and TEM to be near-spherical shape with narrow size distribution. The nanoparticles were further characterized in terms of their size, size distribution, surface charge, drug-loading content, encapsulation efficiency and in vitro drug release profiles. The data showed that the M-PLGA–TPGS nanoparticles were found to be stable, showing almost no change in particle size and surface charge during 3-month storage of their aqueous solution. In vitro Genistein release from the nanoparticles exhibited biphasic pattern with burst release at the initial 4 days and sustained release afterwards. The cellular uptake efficiency of fluorescent M-PLGA–TPGS nanoparticles was 1.25-, 1.22-, and 1.29-fold higher than that of the PLGA–TPGS nanoparticles at the nanoparticle concentrations of 100, 250, and 500 μg/mL, respectively. In the MPTN group, the ratio of apoptotic cells increased with the drug dose increased, which exhibited dose-dependent effect and a significant difference compared with Genistein solution group ($p < 0.05$). The data also showed that the Genistein-loaded M-PLGA–TPGS nanoparticles have higher antitumor efficacy than that of linear PLGA–TPGS nanoparticles and PLGA nanoparticles in vitro and in vivo. In conclusion, the star-shaped copolymer M-PLGA–TPGS could be used as a potential and promising bioactive material for nanomedicine development for liver cancer treatment.

1. Introduction

The applications of nanotechnology in various disciplines and specifically in medicine are becoming increasingly popular and the process of replacing traditional medicines has already begun [1–5]. Liver cancer is one of the most common cancer in the world, and the majority of patients with liver cancer will die within one year as a result of the cancer. There is a substantial interest in developing therapeutic options for treatment of liver cancer based on use of nanoparticle formulations, to overcome the lack of specificity of conventional chemotherapeutic drugs as well as for the early detection of precancerous and malignant lesions. In addition, the nanoparticles used as drug carriers possess other advantages including stable structure, high drug encapsulation efficiency, high cellular uptake, more desirable biodistribution and more reasonable pharmacokinetics as well as preferentially accumulate at the tumor site through the enhanced permeability and retention (EPR) effect [6,7]. Polymeric nanoparticles were also found to be able to reduce or overcome multi-drug resistance (MDR) of cancer cells [8].

Biodegradable polymers have shown great potential for use in drug delivery and tissue engineering. Among them, polyester family including poly(D,L-lactide-co-glycolide) (PLGA), polyglycolide (PGA) and polylactide (PLA) are most extensively studied due to their good biocompatibility and biodegradability [7,9]. Despite the well-established

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importance, such kind of polymers still has limitations in particular applications. It is well known that the autocatalytic effect and the acidic degradation products of these polyesters cause unfavorable effects. In addition, degradation rate of polyesters such as PLA and PLGA is too slow due to its hydrophobic nature to meet the therapeutic needs [10,11]. It was also reported that the PLA- and PLGA-based nanoparticles could be rapidly cleared in the liver and captured by the reticuloendothelial system (RES) when they are delivered into the blood circulation [12,13]. These drawbacks could be overcome by the introduction of d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) into the hydrophobic PLGA backbone [1,2]. TPGS, a water-soluble derivative of the natural form of d-α-tocopherol, is formed by esterification of vitamin E succinate with PEG 1000. It was reported that TPGS was able to improve the aqueous solubility of drugs including taxanes, antibiotics, cyclosporines and steroids. In addition, TPGS could serve as an excellent molecular biomaterial for overcoming MDR and as an inhibitor of P-glycoprotein to increase the cytotoxicity and oral bioavailability of anticancer drugs [1,2].

Although nanoparticles of biodegradable copolymer have been extensively investigated as delivery vehicles of drugs, most of them were focused on making use of linear polymers. In recent years, branched polymers, such as hyper-branched polymers, star-shaped polymers and dendrimers have obtained great attention due to their useful mechanical and rheological properties [7,14,15]. A star-shaped diblock polymer is a branched polymer molecule in which a single branch point (core) gives rise to multiple linear chains or arms [16]. In comparison with the linear polymers at the same molar mass, the nanocarriers based on star-shaped polymer molecule showed a smaller hydrodynamic radius, lower solution viscosity, higher drug content and higher drug entrapment efficiency [17,18]. Therefore, in this research, novel drug delivery systems of star-shaped block copolymers based on PLA and TPGS with unique architectures were developed, which would provide valuable insights for fabricating ideal and useful drug vehicles for nanomedicine applications [19,20]. Mannitol, which is a white crystalline sugar alcohol with the chemical formula ($C_6H_8(OH)_6$) [20,21], does not absorb moisture, is fast drying and exhibits good chemical stability. Thus, it is widely used for tablets of anti-cancer agents. In this research, the star-shaped block copolymer M-PLGA–TPGS with three branch arms was used for developing a superior nanocarrier of anticancer agents with satisfactory drug loading content and entrapment efficiency for liver cancer treatment. The star-shaped M-PLGA–TPGS nanoparticles containing Genistein as a model drug molecule were characterized and the anticancer activity of nanoparticles was evaluated both in vitro and in vivo.

2. Materials and methods

2.1. Materials

D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS), 4′-6′-diamino-2-phenylindole (DAPI), Genistein powder with about 98% purity (HPLC), and PLA (M_w ~25,000) were purchased from Sigma-Aldrich (St. Louis, MO, USA). M-PLGA–TPGS diblock copolymer (M_w ~24,000) and PLGA–TPGS (M_w ~24,000) copolymer was obtained from Tsinghua University Shenzhen Graduate School. All chromatographic solvents were of HPLC grade quality and all other chemicals used were of the highest grade commercially available. Distilled deionized water was used throughout the experiments. Human liver cancer cell line HepG2 was obtained from American Type Culture Collection (ATCC, Rockville, MD).

2.2. Formulation of Genistein-loaded nanoparticles

Genistein-loaded nanoparticles were prepared by a modified nanoprecipitation method as previously described [22]. In short, 10 mg Genistein and 100 mg PLGA polymer, PLGA–TPGS or M-PLGA–TPGS copolymer were dissolved in 8 mL acetone. Then the dissolved mixture was slowly added into 100 mL of 0.03% (w/v) TPGS aqueous solution using 1 mL injector under stirring. After stirring overnight at room temperature to completely remove acetone, the suspension was centrifuged at 25,000 rpm for 15 min and then washed three times to remove the emulsifier TPGS and unencapsulated drug. Finally, the resulting nanoparticles were dispersed in 10 mL water and lyophilized for further use. The preparation of fluorescent coumarin-6-loaded PLGA, PLGA–TPGS and M-PLGA–TPGS NPs was by the same method except for 1 mg coumarin-6 instead of Genistein. The lyophilized nanoparticles were redispersed in PBS before use.

2.3. Characterization of Genistein-loaded nanoparticles

2.3.1. Particle size, surface charge and surface morphology

The nanoparticle size and zeta potential were determined by Malvern Mastersizer 2000 (Zetasizer Nano ZS90, Malvern Instruments Ltd., UK). Before measurement, the freshly prepared nanoparticles were appropriately diluted. All measurements were performed at room temperature after equilibration for 10 min. The data were obtained with the average of three measurements.

The surface morphology of nanoparticles was observed with a field emission scanning electron microscopy (FESEM, JEOL JSM-6301F, Tokyo, Japan). To prepare samples for FESEM, the particles were fixed on the stub by a double-sided sticky tape and then coated with a platinum layer by JFC-1300 automatic fine platinum coater (JEOL, Tokyo, Japan) for 45 s. The morphology of the nanoparticles was also observed by transmission electron microscopy (TEM, Tecnai G2 20, FEI Company, Hillsboro, Oregon, USA). Before observation, the sample was deposited onto a copper grid coated with carbon and dried at room temperature.

2.3.2. Drug loading content and entrapment efficiency

To determine the drug loading (LC) capacity and entrapment efficiency (EE) of the Genistein-loaded nanoparticles, predetermined amount of nanoparticles was dissolved in 1 mL methylene dichloride under vigorous vortexing. The organic solution was transferred to 5 mL of mobile phase. A nitrogen stream was introduced to evaporate the methylene dichloride until a clear solution was obtained. The samples were further monitored by HPLC analysis (LC 1100, Agilent Technologies, Santa Clara, CA, USA). After passing through a 0.45 μm filter to remove precipitation, the clear solution was subjected to HPLC analysis using a reverse-phase C-18 column Methanol/water (60/40, v/v) was used as the mobile phase at a flow rate of 1 mL/min. A reverse-phase C_{18} column (150 × 4.6 mm; pore size, 5 μm; Agilent Technologies) at 25 °C to determine the amount of encapsulated Genistein at 262 nm. Methanol/water (60/40, v/v) was used as the mobile phase at a flow rate of 1 mL/min. The amount of Genistein was calculated according to a standard Genistein sample. The LC and EE of the Genistein-loaded nanoparticles were then calculated.

2.3.3. In vitro drug release

In vitro Genistein release from polymeric nanoparticles was performed as reported previously [22,23]. Briefly, 10 mg of accurately weighted Genistein-loaded nanoparticles was resuspended in 1 mL of releasing buffer (PBS [phosphate-buffered saline] containing 0.1% w/v Tween-80) and transferred into a dialysis tube (MWCO = 3.5 kDa and the dialysis area is 1 cm^2). Subsequently, the dialysis tubes were immersed into a centrifuge tube containing 10 mL of releasing buffer and incubated at 37 °C with gentle shaking (130 × g). At designated time intervals, the entire release buffer was collected and replaced with prewarmed fresh release buffer. The released drug was quantified using HPLC in terms of DL and EE. The accumulative release of Genistein from nanoparticles was plotted against time.