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Development of a folate-modified curcumin loaded micelle delivery system for cancer targeting

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ABSTRACT

Targeted drug delivery system for tumor cells is an appealing platform on enhancing the therapeutic effects and reducing the side effects of the drug. In this study, we developed folate-modified curcumin (Cur) loaded micelles (Cur-FPPs) for cancer chemotherapy. The targeting material, Folate-PEG_{3000}-PLA_{2000}, was synthesized by the amide bond formation reaction. And the Cur loaded micelles were prepared by thin-film hydration method with $mPEG_{2000}$-PLA_{2000} (Cur-PPs) or $mPEG_{2000}$-PLA_{2000} and Folate-PEG_{3000}-PLA_{2000} (Cur-FPPs) as carrier. A central composite design (CCD) was used to optimize the formulation, and the optimized Cur-FPPs was prepared with the weight ratio of Folate-PEG_{3000}-PLA_{2000} and $mPEG_{2000}$-PLA_{2000} at 1:9. The average size of the mixed micelles was 70 nm, the encapsulating efficiency and drug-loading were $80.73 \pm 0.16\%$ and $4.84 \pm 0.01\%$, respectively. Compared with the Cur propylene glycol solution, the in vitro release of Cur from Cur-FPPs showed a sustained manner. Furthermore, the *in vitro* cytotoxicity and cellular uptake of Cur-FPPs were significantly enhanced towards MCF-7 and HepG2 cells. The pharmacokinetic studies in rats indicated that a 3-fold increase in the half-life was achieved for Cur loaded micelle formulations relative to solubilized Cur. All the results demonstrated that folate-modified Cur micelles could serve as a potential nanocarrier to improve the solubility and anti-cancer activity of Cur.

1. Introduction

Curcumin (Cur), bis(4-hydroxy-3-methoxyphenyl)-1,6-diene-3,5-dione, is a polyphenol compound derived from the rhizome of plant *Curcuma longa*. Recently, it has been reported that Cur has a wide range of pharmacologic activities such as anti-inflammation, anti-human immuno-deficiency virus, anti-microbial, anti-oxidant, anti-parasitic, anti-mutagenic and anti-cancer with low or no intrinsic toxicity [1–4]. Among these pharmacologic activities, the anti-cancer activity has attracted the greatest attention of researchers. Cur can prevent and inhibit the generation, vegetation and metastasis of many kinds of tumors, such as breast cancer, cervical cancer, colon carcinoma, stomach cancer, liver cancer, epithelial cell carcinoma, pancreatic cancer [5]. Now the studies on Cur for cancer are still on the status of clinical studies. For example, phase II clinical study is ongoing with Cur for the treatment of pancreatic cancer [6], and the breast cancer studies are on clinical phase I [7]. Despite of the excellent anti-cancer properties, the application of Cur in clinic has been limited because of its low solubility in aqueous solution and rapid degradation in physiological conditions [8,9]. Therefore, it is necessary to improve the stability, solubility and bioactivity of Cur.

Recent studies show that polymeric micelle is one of the most attractive alternatives for hydrophobic drugs to improve their bioavailability. Amphiphilic block copolymers can form nano-sized aggregates with core-shell structure which can solubilize poorly water soluble drugs. Compared with other delivery systems, the polymeric micelles have many advantages as follows [10–13], (i) improving the apparent dissolvability of drugs and protecting them from adverse surrounding environments through entrapping these hydrophobic drugs into hydrophobic inner core, (ii) reducing the nonspecific uptake by the reticuloendothelial system (RES), and thus prolonging the circulation time in blood due to its flexible or hydrophilic outer core, (iii) achieving the possible targeting delivery via surface modification with targeting molecules.

Folate receptor is highly expressed in several human tumor cells including breast, liver, uterus, testis, brain, colon and lung

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cancer cells [14]. Folate and folate conjugates can bind to the folate receptor with high affinity and enter cells by receptor-mediated endocytosis [15], so the folate-drug delivery carriers can transfer the therapeutical agents to tumor cells highly expressing folate receptor. Since the folate receptor-mediated endocytosis was discovered, folate based candidates have been widely used for targeted drug delivery [16]. Studies showed that the uptake of the folate-modified nanoconjugate by HepG2 cells was 12-fold higher than that of non-folate nanoparticles after 4 h incubation [17]. What is more, a folate-modified chitosan micelles, folate-SO-chitosan micelles, were also proved to be promising tumor targeting carriers for hydrophobic anti-tumor agents [14]. Many studies have demonstrated that the folate-drug delivery systems can increase the targeting capability of drugs to the tumor tissue. In the present study, poly(ethylene glycol)-poly(lactic acid) (PEG-PLA), one of biocompatible and biodegradable materials, was used as a delivery carrier to improve the solubility of Cur in aqueous solution. To strengthen the drug's antiangiogenic efficacy and improve the anti-tumor efficacy, Cur-loaded polymeric micelles (Cur-FPPs) based on folate targeting amphiphilic copolymer (Folate-PEG-PLA) were developed. The physicochemical and biological properties such as cellular uptake and the in vitro cytotoxicity of the micelles were evaluated in MCF-7 and HepG2 cells. The pharmacokinetics in vivo was also investigated after an i.v. administration in rat.

2. Materials and methods

2.1. Materials

Cur, folate, 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-diphenytetrazoliumromide (MTT) and coumarin 6 were supplied by Sigma-Aldrich Corporation (St Louis, MO). PLA_{2000}-PEG_{3000}-NHS was purchased from Advanced Polymer Materials Inc. (Canada). $mPEG_{2000}$-PLA_{2000} was obtained from Jinan Daigang Co. Ltd. (Jinan, China). Human breast cancer cell line MCF-7 and human liver hepatocellular carcinoma cell line HepG2 were obtained from Department of immunology of pharmacology, Shandong University School of Medicine (Jinan, China).

2.2. Folate-PEG_{3000}-PLA_{2000} synthesis and characterizations

Based on the previous reports [14,18,19], Folate-PEG-PLA was synthesized as follows, NHS-PEG_{3000}-PLA_{2000} was dissolved into quantitative dimethylformamide (DMF), and then the triethylamine (TEA) and folate were added with magnetic stirring. The reaction mixture was stirred at room temperature for another 24 h. The product was dialyzed against DMSO for 48 h, then dialyzed against water for 48 h, during which the DMSO or water was renewed every 3 h, and finally freeze-dried to obtain Folate-PEG_{3000}-PLA_{2000}. All the procedure was conducted under the dark circumstances.

^{1}H NMR spectra were recorded on a Varian Inova 300 MHz spectrometer. The solvent for NHS-PEG_{3000}-PLA_{2000} and Folate-PEG_{3000}-PLA_{2000} was DMSO-d6.

2.3. Polymer micelle formation

2.3.1. Preparation of Cur loaded mPEG-PLA micelles (Cur-PPs)

Cur-PPs were prepared through film-hydration method [20–23]. A total of 80 mg of $mPEG_{2000}$-PLA_{2000} and 5 mg of Cur were dissolved in 4 mL of methylene bichloride (CH_2Cl_2) in a 25 mL round-bottom flask. The solution was evaporated to form thin film at 37 °C. Residual CH_2Cl_2 remaining in the film was removed under vacuum overnight at room temperature. Then, the resultant thin film was hydrated with 2 mL normal saline. The mixture was stirred at 700 rpm for 30 min to obtain a micelle solution at 37 °C, which was then centrifuged at 4000 rpm for 15 min to remove the unencapsulated Cur.

2.3.2. Preparation of Cur loaded Folate-PEG-PLA micelles (Cur-FPPs)

For Cur-FPPs, similar procedure was taken except that Folate-PEG-PLA was mixed with PEG-PLA. A total of 8 mg of functionalized Folate-PEG_{3000}-PLA_{2000}, 72 mg of $mPEG_{2000}$-PLA_{2000} and 5 mg of Cur were dissolved in 4 mL of CH_2Cl_2, and the followed process was the same as "2.3.1" section.

2.4. Physicochemical characterization of Cur-FPPs

2.4.1. Morphology

The microstructure of Cur-FPPs was observed under the transmission electron microscopy (TEM, JEM-1200EX, JEOL, Tokyo, Japan). In brief, a droplet of micelle suspension was placed on a film-coated copper grid, and then a drop of 1% (w/v) phosphotungstic acid aqueous solution was dropped onto the surface of the sample-loaded grid. Finally, the sample was dried in air and then examined under TEM.

2.4.2. Particle size, zeta potential

The size distribution and zeta potential of micelles in an aqueous medium (the concentration of polymer was 1 mg/mL) were measured using a Delsa™ Nano C Zeta potential/size analyzer (Beckman Coulter Instruments, US).

2.4.3. Entrapment efficiency (EE) and drug loading (DL)

In order to determine the EE and DL of Cur-FPPs, the drug loaded micelle solutions were dissolved in methanol, and analyzed by measuring the absorbance of the resultant solutions at a wavelength of 428 nm using an ultraviolet-visible spectrophotometer (UV-2102, Shanghai Instrument Ltd, China). The EE and DL of Cur-FPPs were calculated according to the following equations,

$$EE\,(\%) = \frac{W_{entrapped}}{W_{total}} \times 100\% \quad (1)$$

$$DL\,(\%) = \frac{W_{entrapped}}{W_{Cur\text{-}FPP}} \times 100\% \quad (2)$$

In above equations, $W_{entrapped}$ showed the amount of Cur entrapped in Cur-FPPs, W_{total} was the feeding drug, and $W_{Cur\text{-}FPP}$ presented the weight of the feeding polymer and drug.

2.5. In vitro drug release

The Cur-FPPs or Cur-PPs solution was put into a dialysis tube (MWCO 3000–5000) and subjected to dialysis against 50 mL normal saline with 1% Tween 80 in dark environment at 37 °C [24] with the Cur propylene glycol solution as control. 1 mL of sample was taken out from the release medium periodically at 0.5, 1, 2, 4, 6, 10, 12, 24, 36, 48, 60, 72 and 96 h. The volume of solution was kept constant by adding 1 mL of fresh normal saline with 1% Tween 80 after each sampling. The drug concentration was determined by HPLC.

2.6. In vitro cytotoxicity and cellular uptake

2.6.1. MTT assay for cell viability

Cells were cultured in folate-free RPMI-1640 (GIBCO company, US) supplemented with 10% fetal bovine serum (FBS), and the cultures were maintained in a fully humidified atmosphere with 5% CO_2 at 37 °C. For dose-dependent cytotoxicity assays, cells were seeded in 96-well plates at 5×10^3 cells/well and pre-incubated for 12 h. The cells in each well were cultured in 200 μL of folate-free RPMI-1640 medium with 10% FBS. Then the culture medium