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Research paper

Pharmacokinetics and in vivo delivery of curcumin by copolymeric mPEG-PCL micelles

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ABSTRACT

Curcumin (CUR) has been associated with anti-inflammatory, antimicrobial, antioxidant, anti-amyloid, and antitumor effects, but its application is limited because of its low aqueous solubility and poor oral bioavailability. To progress the bioavailability and water solubility of CUR, we synthesized five series of mono methoxy poly (ethylene glycol)-poly (ε-caprolactone) (mPEG-PCL) diblock copolymers. The structure of the copolymers was characterized by H NMR, FTIR, DSC and GPC techniques. In this study, CUR was encapsulated within micelles through a single-step nano-precipitation method, leading to formation of CUR-loaded mPEG-PCL (CUR/mPEG-PCL) micelles. The resulting micelles were characterized further by various techniques such as dynamic light scattering (DLS) and atomic force microscopy (AFM). The cytotoxicity of void CUR, mPEG-PCL and CUR/mPEG-PCL micelles was compared to each other by performing MTT assay of the treated MCF-7 and 4T1 cell line. Study of the in vivo pharmacokinetics of the CUR-loaded micelles was also carried out on selected copolymers in comparison with CUR solution formulations. The results showed that the zeta potential of CUR-loaded micelles was about −11.5 mV and the average size was 81.0 nm. CUR was encapsulated into mPEG-PCL micelles with loading capacity of 20.65 ± 0.015% and entrapment efficiency of 89.32 ± 0.34%. The plasma AUC (0–t), $t_{1/2}$ and C_{max} of CUR micelles were increased by 52.8, 4.63 and 7.51-fold compared to the CUR solution, respectively. In vivo results showed that multiple injections of CUR-loaded micelles could prolong the circulation time and increase the therapeutic efficacy of CUR. These results suggested that mPEG-PCL micelles would be a potential carrier for CUR.

1. Introduction

Cancer is the important cause of death in industrial countries and the second-leading reason of death in developing countries [1]. Natural products derived from plants play a significant function in the health care of many cultures, both ancient and modern [2–6]. Currently, over 100 new natural products are in clinical development, particularly anticancer and anti-infective agents [2]. Natural compounds with antitumor activity and partial toxicity are receiving more and more notice in medical research [3]. Curcumin (CUR), a chemical constituent of turmeric, has been applied in the treatment of inflammatory disorders and cancer for a lot of years [4]. There is some data signifying that CUR is a principle chemo sensitizer for chemotherapy and that it helps to care for patients from the side effects of treatment [5–10]. CUR may reduce tumor growth via multiple mechanisms, counting antitumor angiogenesis [11,12], suppression of proliferation [13,14], induction of apoptosis [7,15] and prevention of metastasis [16,17]. However, the clinical applications of CUR remain limited because of its short biological half-life, poor solubility resulting in poor absorption, and low bioavailability via the oral route [18–20]. Development of an intravenous preparation is a promising approach to resolving these issues in the application of CUR [21]. Nanotechnology has the prospective to defeat the poor water solubility of lipophilic drugs. Encapsulation of hydrophobic drugs into nanoparticles is a hopeful advance to formulate the drug

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intravenously injectable [22]. The amphiphilic nanoparticles that include a hydrophobic core and a hydrophilic shell are excellent candidates for carrying hydrophobic drugs. mPEG-PCL is a diblock PCL-PEG copolymer that self-assembles into nanoparticles with core–shell structure: a hydrophobic PCL core and a hydrophilic PEG shell. These copolymers are nontoxic and not accumulative in vivo because the degradation products of mPEG–PCL block copolymer can enter the tricarboxylic acid cycle or be eliminated by the kidney. This copolymers can increase the drug loading, reduce the burst effect, prolong the in vivo residence time of drugs, avoid them being engulfed by macrophages, improve hydrophilicity, degradation rate, crystallization and show great potential for development in drug delivery [23]. Although encapsulated with the hydrophobic drug, the hydrophobic PCL segment joint with the drug forms the core and the hydrophilic PEG forms the shell of nanoparticles, assembly the drug intravenously injection. In addition to entrapment with hydrophobic drugs mPEG-PCL copolymers possibly will improve constancy and systemic distribution of drugs and release drugs at a sustained time in the finest variety of drug concentration. Furthermore, mPEG-PCL copolymers are recyclable, biocompatible, and simple to construct, showing hopeful applications in drug-delivery systems [23,24]. Newly mPEG-PCL micelles were applied to carry hydrophobic drugs (such as sulforaphane), with the aim of bringing narrative aqueous formulations for these drugs [23,24]. In this study, in an effort to support an aqueous formulation for CUR, we organized CUR encapsulated mPEG-PCL micelles. Our outcome showed that encapsulation of CUR in mPEG-PCL micelles rendered CUR completely dispersible in water. Also, CUR/mPEG-PCL micelles were able to slowly release CUR and professionally restrain the expansion of cancer cells in vitro and in vivo. These records recommended that CUR/mPEG-PCL micelles can be a narrative nanoformulation of CUR with capable purpose in cancer therapy.

2. Materials and methods

2.1. Materials

1-(4,5-Dimethylthiazol-2-yl)-3,5-diphenylformazan, Thiazolyl blue formazan (Aldrich, St. Louis, USA, CAS. 57360-69-7), mPEG (Mn = 5000 Da) (Aldrich, St. Louis, USA, CAS.81323), 2-ethylhexanoate ($Sn(Oct)_2$) (Aldrich, St. Louis, USA, CAS. 301100), ε-caprolactone (98% purity) (Acros, New Jersey, USA, CAS.502443), CUR (Merck, Darmstadt, Germany, Art No. 820354), and other chemicals and solvents were from chemical lab purity grades and purchased from Emertat chimi (Emertat, Iran).

2.2. Synthesis of mPEG–PCL copolymers

mPEG-PCL diblock copolymers were prepared according to our previous report [25] and that were synthesized by a ring opening polymerization of ε-caprolactone with mPEG as initial molecule and $Sn(Oct)_2$ as catalyst. In brief, ε-caprolactone (0.5, 1, 2, 4, 5, 6 g), mPEG (1 g), and $Sn(Oct)_2$ (0.01 mmol) were heated to 120 °C to begin polymerization. Later than 12 h, the resultant copolymers were cooled to room temperature, dissolved in chloroform, and precipitated in cold diethyl ether. The copolymer was dried up under vacuum at room temperature for 24 h.

2.3. Characterization of mPEG-PCL copolymers

The chemical structure of copolymers was known by Fourier transform infrared spectroscopy (FT-IR) (Bruker, Tensor 27, Biotage, Germany) and proton nuclear magnetic resonance spectroscopy (H NMR) in $CDCl_3$ at 400 MHz (Bruker, Avance 400, Munich, Germany). Differential scanning calorimetry (DSC) (Mettler Toledo, model Star SW 9.30, Schwerzenbach, Switzerland) was applied for thermal analysis of the synthesized copolymers. Samples were heated at a rate of 10 °C min^{-1} and the records were recorded from 0 to 200 °C. The average molecular weight and distribution of the mPEG–PCL copolymers were indomitable by gel permeation chromatography (GPC) (Knauer, Berlin, Germany) set with differential refractometric detector and an ultrastyra gel column (4.6 × 30 mm) (Waters, Milford, USA, model HR 4E). The mobile phase was tetrahydrofuran (THF) with a flow-rate of 1 mL/min and the injection volume was 50 μL of stock solutions (0.1–0.5 w/v %). Polymers were characterized by relative elution time to polystyrene monodisperse standards in the range of 1500–35,500 Da (Varian Palo Alto, CA, USA) using the calibration curve obtained previous to measurements.

2.4. Preparation of CUR-loaded micelles

CUR-loaded micelles were prepared according to our previous report [26,27]. Nanoprecipitation method was used for preparation of micelles. The acetone was used as the solvent to this method. In brief, mPEG–PCL copolymer (20 mg), and CUR (1, 2, 5, 10, 15, 20 mg) were dissolved in 2 mL of acetone. The solution was, then, injected dropwise during a syringe (G = 22) into 25 mL of distillated water under certain combination rates and stirred magnetically at room temperature awaiting whole disappearance of the organic solvent which caused the amphiphilic copolymers to self-assemble to form the micelles. After removing of acetone by rotary vacuum evaporation at 35 °C, the resultant yellowish aqueous solution was filtered throughout a 0.45 μm filter membrane to eliminate the unloaded CUR. The resulting molecules by centrifuging at 20,000*g* for 20 min were separated and freeze-dried under a pressure of 14 Pa at −78 °C until to separate all the residual solvents and to obtain the final dried form of micelles loaded by CUR.

2.5. Characterization of the micelles

2.5.1. Particle morphology

The morphology of micelles was assigned using atomic force microscopy (AFM) (JPK, Berlin, Germany, model Nano Wizard). For AFM sample preparation, micelles were diluted with water and a droplet of 2 μL was placed onto a freshly cleaved mica substrate (1 cm^2) and air-dried. AFM measurements were performed in intermittent contact mode.

2.5.2. Determination of particle size

The particle size distribution of the organized micelles was assigned by dynamic light scattering (DLS) using a nano/zetasizer (Malvern Instruments, Worcestershire, UK, model Nano ZS).

2.5.3. Stability of micelles

For evaluation of physical stability of micelles the particle size distribution of the micelles was monitored in phosphate-buffered solution (PBS, pH = 7.4) and kept in room temperature at times 0, 15, and 30 days after grounding using the method described in the above section.

2.5.4. Determination of loading efficiency

Two parameters including the drug loading ratio and efficiency of entrapment were determined for determination of the loading efficiency of the drugs in the micelles. Drug loading ratio was determined as follows:

$$\%DL = \frac{\text{weight of drug in micelles}}{\text{weight of micelles}} \times 100 \quad (1)$$