International Journal of Pharmaceutics 507 (2016) 32–40

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Characteristics and cytotoxicity of folate-modified curcumin-loaded PLA-PEG micellar nano systems with various PLA:PEG ratios

Quoc Thong Phan[a,b], Mai Huong Le[c], Thi Thu Huong Le[a], Thi Hong Ha Tran[c], Phuc Nguyen Xuan[a], Phuong Thu Ha[a,*]

[a] Institute of Materials Science, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Cau Giay District, Ha Noi City, Vietnam
[b] University of Khanh Hoa, 01 Nguyen Chanh Road, Nha Trang City, Khanh Hoa Province, Vietnam
[c] Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Cau Giay District, Ha Noi City, Vietnam

ARTICLE INFO

Article history:
Received 15 February 2016
Received in revised form 11 April 2016
Accepted 2 May 2016
Available online 3 May 2016

Keywords:
Curcumin (Cur)
PLA-PEG copolymer
PLA-PEG/Cur
PLA-PEG/Cur/Fol

ABSTRACT

Targeting delivery system use natural drugs for tumor cells is an appealing platform help to reduce the side effects and enhance the therapeutic effects of the drug. In this study, we synthesized curcumin (Cur) loaded (D, L Poly lactic – Poly ethylenglycol) micelle (Cur/PLA-PEG) with the ratio of PLA/PEG of 3:1 2:1 1:1 1:2 and 1:3 (w/w) and another micelle modified by folate (Cur/PLA-PEG-Fol) for targeting cancer therapy. The PLA-PEG copolymer was synthesized by ring opening polymerization method. After loading onto the micelle, solubility of Cur increased from 0.38 to 0.73 mg ml^{-1}. The average size of prepared Cur/PLA-PEG micelles was from 60 to 69 nm (corresponding to the ratio difference of PLA/PEG) and the drug encapsulating efficiency was from 48.8 to 91.3%. Compared with the Cur/PLA-PEG micelles, the size of Cur/PLA-PEG-Fol micelles were from 80 to 86 nm and showed better in vitro cellular uptake and cytotoxicity towards HepG2 cells. The cytotoxicity of the NPs however depends much on the PEG component. The results demonstrated that Folate-modified micelles could serve as a potential nano carrier to improve solubility, anti-cancer activity of Cur and targeting ability of the system.

1. Introduction

Curcumin (Cur) or diferuloylmethane, bis(4-hydroxy-3-methoxyphenyl)-1,6-diene-3,5-dion is a yellow polyphenol compound extracted from the rhizome of turmeric (*Curcumin longa*), a plant grown in tropical Southeast Asia (Shishodia et al., 2005). Recently, a great deal of research 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 (El-Moselhy et al., 2011; Srivastava et al., 2011; Akhand et al., 2001). Among these pharmacologic activities, Cur has been paid most attention on its anti-cancer activity to prevent and inhibit the generation, metastasis of many of kinds of tumors, such as breast cancer, cervical cancer, colon carcinoma, stomach cancer, liver cancer, epithelial cell carcinoma, pancreatic cancer (Banerji et al., 2004). The clinical studies of Cur for cancer are still continuous. For example, the treatment of pancreatic by Cur is studied on phase II clinical (Kim et al., 2011), and studied on phase I clinical for breast cancer (Dhillon et al., 2008). Despite of its excellent anti-cancer properties, low solubility in aqueous solution and rapid decomposition in physiological conditions of Cur make it become limited in clinical application (Bayet-Robert et al., 2010; Wu et al., 2011). Therefore, improving the stability, solubility and bioactivity of Cur is necessary.

Among the numerous ways to improve bioavailability of hydrophobic drugs, using polymeric micelle is one of the most attractive alternatives. The polymeric micelles have core-shell structure formed by amphiphilic block which can solubilize water poorly soluble drugs. The polymeric micelles have such more advantages than other delivery systems as: (Jurenka, 2009; Chen et al., 2008; Xiao et al., 2012; Huh et al., 2005), (i) able to conjugate with targeting molecules via surface modification and achieve the possible targeting, (ii) capturing the hydrophobic drugs into hydrophobic inner core so that protecting drugs from adverse surrounding environments and improving the apparent

Abbreviations: Cur, Curcumin; DCM, dichlomethan; DMSO, dimethylsulfoxide; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; Fol, Folate; HepG2, Human liver hepatocellular carcinoma cell line; NHS, hydroxysuccinimide; NP, nanoparticle; PBS, phosphate buffered saline; RES, reticuloendothelial system.

* Corresponding author at: Head of Lab Biomedical Nanomaterials, Institute of Materials Science, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Road, Cau Giay District, Ha Noi City, Vietnam.
E-mail addresses: thuhp@ims.vast.ac.vn, haphuongthu74@gmail.com (P.T. Ha).

http://dx.doi.org/10.1016/j.ijpharm.2016.05.003
0378-5173/

dissolvability of drugs, (iii) reducing the nonspecific uptake by the reticuloendothelial system (RES).

In addition, using active targeting ligands offers higher effectiveness for nano drug delivery. Targeting ligands may be antibodies, aptamer, peptides or small molecules. Among them, folic acid, a small molecule is required for essential cell function, has the ability to link closely with highly expression folate receptor on the surface of many cancer cells including breast, kidney, lung, brain, and ovary cancers (Parkera et al., 2005). Folate-drug delivery systems can enter cells by receptor-mediated endocytosis which can avoid their non-specific attacks to normal tissues as well as increase their cellular uptake within target cells and transfer the therapeutic agents to tumor cells (Behdokht et al., 2015). It is reported that folate conjugation can increase the stability of FA-copolymer micelle compared to copolymer (PLA-MPEG) only, which was in part due to the lower micelle concentration (Zhu et al., 2011a, 2011b). Similarly, it is demonstrated that folate decorated, curcumin loaded nanoparticle could enhancing therapeutic potential of Nutlin-3a by reversing multidrug resistance (Das and Sahoo, 2012). Our previous investigations also showed that the folate-drug delivery systems can increase the targeting capability and uptake of drugs to the tumor cells (Ha et al., 2015) as well as prolong the retention time in the body (Huong et al., 2016). In this study, poly(lactic acid)-poly(ethylene glycol) was used to develop a folate-modified curcumin loaded micelle delivery system (Cur/PLA-PEG-Fol) to improve solubility of curcumin in aqueous solution and increase targeting ability of curcumin. The cellular uptake and *in vitro* cytotoxicity of the micelle were evaluated in HepG2 cells.

2. Material and methods

2.1. Material

Curcumin (Cur), lactic acid (LA), polyethylen glycol (PEG 2000), stannous octoate ($Sn(Oct)_2$), maleic anhydride, folic acid were purchased from Sigma (USA). *N*-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), Solvents (toluene, dichlomethan (DCM), methanol, ethanol, dimethylsulfoxide (DMSO), phosphate buffered saline PBS (pH 7.4)) were purchased from Merck (Darmstadt, Germany). Human liver hepatocellular carcinoma cell line HepG2 was supported from Institute of Natural Products Chemistry – Vietnam Academy of Science and Technology (Hanoi, Vietnam). All chemicals were used without further purification. Distilled water was used for all experiments.

2.2. Methods

2.2.1. Synthesis of PLA-PEG block copolymers

5 PLA-PEG block copolymers were synthesized by ring-opening polymerization of lactide monomer in the presence of polyethylene glycol (PEG) using stannous octoate as catalyst (Sabharanjak and Mayor, 2005) with the different ratios of PLA/PEG (w/w) (3:1, 2:1, 1:1, 1:2 and 1:3). Polymerization reactions were performed at 130 ?°C under inert gas atmosphere. After 10 h reaction, solvent was evaporated at 110 °C. Obtained copolymer was dissolved in DCM and then purified by precipitating in cool methanol. Purification process was repeated three times and the copolymer was dried under vacuum at 45 °C for 48 h.

2.2.2. Preparation of folate-conjugated PLA-PEG copolymer

First, 3:1 PLA-PEG copolymer solution was prepared by dissolving 80 mg of the copolymer in 80 ml distilled water and magnetically stirring for 6 h. Then maleic anhydride (4 mg) was added to the solution and the reaction mixture was stirred for 6 h at 80 °C in order to form PLA-PEG-COOH which was then activated by adding EDC (5 mg) and NHS (4 mg) in 1 ml of DMSO. The reaction was allowed to complete for 4 h at room temperature. Finally, folic acid (with a $—NH_2$ group in each molecule) was allowed to react with activated PLA-PEG-COOH for 24 h at room temperature to synthesize PLA-PEG-Fol.

The process is performed similarly to the PLA-PEG copolymer with other different ratios of PLA/PEG (2:1, 1:1, 1:2 and 1:3).

2.2.3. Preparation of curcumin loaded polymeric micelles (Cur/PLA-PEG and Cur/PLA-PEG-Fol)

Curcumin loaded PLA-PEG micelles were prepared by emulsification/solvent evaporation method. In brief, Curcumin was dissolved in ethanol and added to PLA-PEG aqueous solutions (1 mg/ml) with gentle stirring at room temperature. After 24 h stirring, ethanol was evaporated and the obtained mixture was centrifuged at 3000 rpm for 10 min to remove the excess curcumin. The excess curcumin was dissolved in ethanol to determine the Curcumin entrapped efficiency.

Cur/PLA-PEG-Fol micelles were synthesized in a similar procedure for Cur/PLA-PEG except PLA-PEG was replaced by a mixture of PLA-PEG-Fol and 5% PLA-PEG.

The process is performed similar to the PLA-PEG copolymer with the difference ratios of PLA/PEG (3:1, 2:1, 1:1, 1:2 and 1:3) (Fig. 1).

2.2.4. Characterization methods

The microstructure the micelles were determined by UV–vis spectra recorded by an ultraviolet-visible spectrometer (UV–vis Aligent 8453) and fluorescence spectra recorded on. Molecular structure of the materials was characterized by Fourier transform infrared spectroscopy (FTIR, SHIMADZU spectrophotometer) using KBr pellets in the wave number region of 400–4000 cm^{-1}. Surface morphology and particle size of materials was investigated by field emission scanning electron microscopy (FE-SEM) on a Hitachi S-4800 system. The amount of curcumin untrapped to the micelles was evaluated by UV–vis spectroscopy at the wave length of 428 nm.

2.2.5. Curcumin entrapment efficiency (EE)

Curcumin EE of Cur/PLA-PEG-Fol was calculated by following formula:

$$EE(\%) = \frac{W_{total} - W_{unentrapped}}{W_{total}} \text{ x } 100$$

In which, W_{total} was the feeding curcumin, $W_{unentrapped}$ showed amount of excess curcumin.

2.2.6. Cur/PLA-PEG-Fol in vitro curcumin release

10 mg of Cur/PLA-PEG or Cur/PLA-PEG-Fol NPs was dispersed in 30 ml PBS (pH 7.4). The dispersion was incubated in water bath at 37 °C. At desired time intervals, 2 ml sample was withdrawn and replaced with an equal volume of the fresh release medium. The curcumin concentration in each taken sample was determined by UV–vis spectroscopy.

2.2.7. In vitro cytotoxicity study

The cytotoxicity of free curcumin, curcumin loaded PLA-PEG polymeric and curcumin loaded PLA-PEG-Fol polymeric with different w/w ratio of PLA and PEG were measured against HepG2 cells using methods reported by Skehan et al. (1990) and Likhitwitayawuid et al. (1993). HepG2 cells were cultured on 96-well plates at concentration of 5×10^3 cells per ml of DMEM (Dulbecco's Modified Eagle Medium) containing 10% BCS (Bovine Calf Serum) and 1% penicillin-streptomycine at 37 °C in an