Colloids and Surfaces B: Biointerfaces 128 (2015) 165–171

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Cysteine modified and bile salt based micelles: Preparation and application as an oral delivery system for paclitaxel

Wei Xu [a], Xiaohui Fan [b], Yanli Zhao [b], Lingbing Li [b,*]

[a] Department of Pharmacy, Shandong Provincial Qian Foshan Hospital, Jinan, Shandong Province, China
[b] Department of Pharmaceutics, School of Pharmaceutical Sciences, Shandong University, Jinan, Shandong Province 250012, China

ARTICLE INFO

Article history:
Received 15 December 2014
Received in revised form 11 February 2015
Accepted 13 February 2015
Available online 21 February 2015

Keywords:
Cysteine modified
Sodium cholate
Micelles
Paclitaxel
Oral

ABSTRACT

The aim of the present study is to construct a cysteine modified polyion complex micelles made of Pluronic F127-chitosan (PF127-CS), Pluronic F127-cysteine (PF127-cysteine) and sodium cholate (NaC) and to evaluate the potential of the micelles as an oral drug delivery system for paclitaxel. Systematic studies on physicochemical properties including size distribution, zeta-potential and morphology were conducted to validate the formation of micelle structure. Compared with Pluronic micelles, drug-loading capacity of PF127-CS/PF127-cysteine/NaC micelles was increased from 3.35% to 12.77%. Both the critical micelle concentration and the stability test confirmed that the PF127-CS/PF127-cysteine/NaC micelles were more stable in aqueous solution than sodium cholate micelles. Pharmacokinetic study demonstrated that when oral administration the area under the plasma concentration–time curve ($AUC_{0-\infty}$) and the absolute bioavailability of paclitaxel-loaded micelles were five times greater than that of the paclitaxel solution. In general, PF127-CS/PF127-cysteine/NaC micelles were proven to be a potential oral drug delivery system for paclitaxel.

1. Introduction

Paclitaxel (PTX) is an effective anti-tumor drug extracted from *Taxus chinensis* and widely used to treat tumors in clinic such as ovarian cancer, breast cancer and colorectal cancer. However, in clinical treatment, the traditional application of PTX is extremely limited to parenteral administration. Oral bioavailability of PTX is even less than 10% [1] because of the poor solubility (about 300 ng/mL), the action of the multidrug efflux transporter P-glycoprotein (P-gp) highly expressed in intestinal tract as well as the extensive first-pass metabolisms by either the intestinal or liver cytochrome P450 enzymes like CYP3A4 [2].

In the last years, many strategies have been contributed in exploring an alternative oral delivery system for PTX not only to improve its poor solubility and low permeability across the intestinal barrier but also overcome the multidrug resistance [3–5]. For example, Yoncheva et al. established stabilized PTX-loaded Pluronic micelles and achieved a higher $AUC_{0-\infty}$ area and longer mean residence time when oral administration, indicating an efficient oral absorption of PTX [6].

Pluronic block copolymers consist of ethylene oxide (EO) and propylene oxide (PO) blocks with a basic EOx–POy–EOx structure. Pluronic block copolymers are benign and some of them have been approved by FDA as pharmaceutical excipients [7]. These macromolecules are surface active and self-assemble into micelles above critical micelle concentrations (CMC) and certain temperatures. Interestingly, Pluronic unimers existing at concentrations below their CMC incorporate themselves into the cell membranes thereby altering the membrane microviscosity and inhibiting functioning of some membrane proteins such as P-glycoprotein (P-gp) responsible for pumping chemotherapeutic drugs out of the cell and other key elements responsible for multidrug resistance in the tumorous cells [8]. However a problem encountered with Pluronic micelles for drug delivery of paclitaxel is its low drug loading.

Cholic acid (CA) is one of the major bile acids produced in the human liver. Its unique facial amphiphilicity makes it a very useful building block for synthesizing biocompatible polymers for drug delivery. Oligomers of CA have been employed as functional molecular containers for caging hydrophobic molecules; however, since low aggregation number due to the steric hindrance of the large steroidal skeleton, their pharmaceutical application is very limited [9]. Many strategies have been contributed to increase the aggregation number of CA. Most of the investigations studied the application of mixed micelles such as phospholipids/bile salts mixed micelles as drug delivery systems [10].

* Corresponding author. Tel.: +86 531 88382015; fax: +86 531 88382548.
E-mail address: cnlilingbing@yahoo.com (L. Li).

http://dx.doi.org/10.1016/j.colsurfb.2015.02.031
0927-7765/

During the last decades diverse materials were synthesized by reacting the block ionomers with oppositely charged molecules such as synthetic surfactants/lipids, proteins, or DNA etc. These materials can form micelle-like nanoparticles in aqueous solution with the core–corona structure called "polyion complex micelles". Surrounded by a hydrophilic corona, the core is formed by the aggregates of surfactant molecules electrostatically bound to the polyion chain. And the pharmaceutical agents can be incorporated into the core through a combination of electrostatic, hydrophobic, and hydrogen bonding interactions. The size, structure, and loading capacity of the core can be altered by changing the ratio of the polyion and surfactant components in the mixture. As a result these systems have potential as drug delivery systems, especially for hydrophobic drugs [11,12].

Thiolated polymers are supposed to be a promising tool in the development of drug delivery systems. Attaching thiol groups on polymers results in tremendous changes in mucoadhesive, cohesive, enzyme inhibitory and permeation enhancing properties. Bernkop-Schnurch and coworkers have demonstrated that the thiolation of bioadhesive delivery systems substantially increases their mucoadhesive property, and therefore, further improves the oral absorption of therapeutic proteins [13]. Werle and Hoffer reported a significantly improved transmucosal transport of P-gp substrate rhodamine-123 in the presence of thiolated chitosan as a result of P-gp efflux pump inhibition [14].

In present work, Pluronic F127-chitosan copolymer (PF127-CS) was synthesized and a type of polyion complex micelles made of PF127-CS and sodium cholate (NaC) was constructed through the electrostatic interaction between PF127-CS and NaC. Pluronic F127-cysteine (PF127-cysteine) was also synthesized and incorporated into formulation to improve the oral absorption. The potential of this kind of micelles as an oral drug delivery system for paclitaxel was evaluated. Systematic studies on physicochemical properties including size distribution, zeta-potential and morphology were conducted to validate the formation of micelle structure. CMC, drug loading content and release behavior were then studied. The pharmacokinetic study in rats was conducted and the oral bioavailability of the micelles was estimated and compared with PTX solution.

2. Materials and methods

2.1. Materials

Paclitaxel (PTX) was purchased from Chengdu Furunde Industrial Co. Ltd. (Chengdu, China). Pluronic F127 (PF127), sodium cholate (NaC), *N*-hydroxysuccinimide (NHS) and *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDC) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Chitosan (Mw ≤3000) was purchased from Jinan Haidebei Marine Bioengineering Co. Ltd. (Jinan, China). Succinic anhydride, DMAP, and triethylamine were purchased from Aladdin Chemical Reagent Co. Ltd. (Shanghai, China). All other reagents and buffer solution components were analytical grade preparation. Distilled and deionized water were used in all experiments.

2.2. Synthesis of PF127-CS copolymers

PF127-CS copolymer was synthesized using succinic anhydride as a bridge molecule between Pluronic F127 and chitosan. Firstly, the carboxylation of terminal hydroxyl groups in Pluronic was synthesized. 3.81 g of Pluronic (0.3 mmol), 45 mg of succinic anhydride (0.45 mmol), 55 mg of DMAP (0.45 mmol), and 60 mg of triethylamine (0.6 mmol) were dissolved in anhydrous dioxane (30 mL) and stirred for 24 h at room temperature. Then, the solvent was removed with a rotary evaporator. The residue was purified by precipitating in diethyl ether twice and dried under vacuum overnight to give white powder of mono-carboxy Pluronic (MP). PF127-CS copolymer was prepared by grafting the MP onto the chitosan. The MP obtained above was mixed with chitosan (Mw ≤3000, 1.35 g, 0.45 mmol), EDC (87 mg, 0.45 mmol) and NHS (52 mg, 0.45 mmol) in 30 mL of PBS buffer (pH 4–5) and stirred at room temperature for 24 h. The resulting mixture was dialyzed against distilled water for 3 days and lyophilized to give PF127-CS [15].

The structures of chitosan, Pluronic F127 and PF127-CS copolymer were confirmed by Fourier transform infrared (FT-IR). Dried samples were pressed with KBr powder into pellets. FT-IR spectra were obtained on FT-IR spectroscopy (Thermo Electron Scientific Instruments Corp., Fitchburg, WI, USA).

2.3. Synthesis of PF127-cysteine polymer

Firstly, the carboxylation of terminal hydroxyl groups in Pluronic was synthesized. Pluronic (0.3 mmol, 3.81 g), succinic anhydride (90 mg, 0.9 mmol), DMAP (110 mg, 0.9 mmol), and triethylamine (120 mg, 1.2 mmol) were dissolved in anhydrous dioxane (30 mL) and stirred for 24 h at room temperature. Then, the solvent was removed with a rotary evaporator. The residue was purified by precipitating in diethyl ether twice and dried under vacuum overnight to give white powder of carboxy Pluronic (CP).

PF127-cysteine copolymer was synthesized by incorporating cysteine to the terminal carboxyl groups in CP. The CP obtained above was mixed with cysteine (142 mg, 0.9 mmol), EDC (176 mg, 0.9 mmol) and NHS (104 mg, 0.9 mmol) in PBS buffer (pH 6) (30 mL) and stirred at room temperature for 3 h. The resulting mixture was dialyzed against distilled water for 3 days and lyophilized to give PF127-cysteine [16].

2.4. Determination of thiol group content

The total amount of thiol groups attached to the thiolated copolymer was spectrophotometrically quantified by Ellman's reagent (DTNB) as described previously [16].

In brief, 1 mg of each conjugate was dissolved in 1 mL of 0.5 M phosphate buffer (pH 8.0). Then 1 mL of 0.03% (m/v) DTNB (DTNB dissolved in 0.5 M phosphate buffer pH 8.0) was added. After incubation for 2 h at room temperature, aliquots of 1 mL was transferred to the cuvette and absorbance at 405 nm was measured. The amount of thiol groups was calculated using a standard curve. The standard curve was established using a series of solutions containing unmodified polymer and increasing amounts of cysteine.

2.5. Preparation of drug-loaded micelles

To prepare drug-loaded PF127-CS/NaC polyion complex micelles, 20 mg of drug and 100 mg of copolymers composed of different amounts of PF127-CS and NaC were dissolved in chloroform (4 mL) and the organic solvent was subsequently removed by rotary vacuum evaporation. The film formed was additionally freeze-dried in vacuum, hydrated with a suitable amount of 5 mM HEPES-buffered saline (HBS). The resulting mixture was filtered through a 0.22 μm nylon filter. The final samples were freeze dried and drug loading content was determined. The drug-loaded PF127-CS/PF127-cysteine/NaC micelles were also prepared by the method described above except that the materials were composed of the mixture of PF127-CS and NaC (100 mg, 80 wt%) and PF127-cysteine (11 mg, 10 wt%).