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The construction and characterization of hybrid paclitaxel-in-micelle-in-liposome systems for enhanced oral drug delivery

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ABSTRACT

In this study, novel paclitaxel (PTX) loaded hybrid liposomes for oral PTX delivery were prepared through incorporating PTX loaded polyion complex micelles comprised of positively charged Pluronic F127-Polyethylenimine (PF127-PEI) copolymer and negatively charged sodium cholate (CA) into liposomes consisted of phospholipid molecules. According to the results, this kind of PTX-loaded hybrid liposomes showed improved PTX encapsulation efficiency, sustained PTX release, and enhanced PTX absorption in intestine. The mechanism for enhancing absorption was demonstrated in connection with inhibition of the efflux mediated by multidrug resistance protein, intestinal P-gp. In pharmacokinetic study, the absolute oral bioavailability of PTX loaded in hybrid liposomes had reached to 37.91%. All of these results demonstrated that the application of this novel PTX loaded hybrid liposomes is a strategy with great potential for highly effective oral PTX delivery.

1. Introduction

Nowadays, cancer has become one of the main reasons resulting in human death. In order to completely eliminate threats brought by cancer, the discovery and production of medicines with high anticancer efficiency will be a continuously concerned field in next few decades. Paclitaxel (PTX) is a kind of naturally sourced highly effective antitumor compound. In clinical, the dosage forms of PTX, for example, Taxol® and Abraxane®, are widely applied in the therapy of solid tumors such as breast cancer, ovarian cancer, non-small cell lung cancer, gastric adenocarcinoma and head and neck cancer [1]. However, there are still some problems greatly limiting the extensive application of PTX and its related dosage forms, especially its low water solubility and low bioavailability caused by the multidrug resistance (MRP) mediated by P-glycoprotein (P-gp) [2,3]. Nowadays, more and more attention has been concentrated on the development of formulations of PTX with high PTX delivery efficiency and low adverse effects and PTX delivery methods with high safety and convenience. Oral drug delivery, because of its high compliance, has been regarded as a widely accepted method for the delivery of drugs for the treatment of serious diseases such as cancer [4]. However, as the result of the poor solubility (0.25 mg/mL) and low permeability across the intestinal barrier caused by the multidrug resistance mediated by P-glycoprotein (P-gp), the oral bioavailability of PTX is even less than 10% [5]. Thus many efforts have been taken to develop an alternative oral delivery system for PTX to improve its poor solubility and low permeability across the intestinal barrier as well as overcome the multidrug resistance against it [5,6].

Polymeric micelles are a kind of nanosized drug carriers with core-shell structure consisted of amphiphilic polymers, hydrophobic drug molecules can be entrapped in hydrophobic core of micelles consisted of hydrophobic moieties of amphiphilic polymer while hydrophilic shell can make micelles disperse in aqueous solution [7]. Encapsulating drugs with low water solubility in polymeric micelles is a promising strategy to improve the solubility of hydrophobic drugs. Recently, different types of PTX loaded polymeric micelles have been in clinical trial or entered into market, such as Genexol®-PM, PTX loaded polymeric micelles composed of monomethoxy poly(ethylene glycol)-block-poly (D,L-lactide) (mPEG-PDLLA) [8].

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Polyion complex micelles are a category of polymeric micelles formed through the self-assembling of oppositely charged polymers under the driving of electrostatic interactions. Compared with conventional polymeric micelles, polyion complex micelles are capable of encapsulating both charged and neutral constituents. The physicochemical properties of polyion complex micelles can vary as the variation of the aqueous environment especially the variation of pH for the reason that the variation of pH value of aqueous solution can influence the ionization degree of charged carrier materials. And the low stability of polyion complex micelles in gastrointestinal fluid greatly limits the oral application of polyion complex micelles [9]. Liposomes are water dispersed nanoparticles with bilayer membrane arranged by amphiphilic lipid molecules such as the molecules of phospholipids. The hydrophobic drug can be incorporated into bilayer membrane and hydrophilic drug can be incorporated into the water soluble central compartment of liposomes [10]. Liposomes show high biocompatibility and low toxicity, and can promote the transmembrane transport of loaded constituents [11]. The encapsulation of drug loaded polyion complex micelles into liposomes can not only improve the drug loading capability of the whole carrier, but can also protect micelles from being degraded in gastrointestinal fluid. In conclusion, the combination of liposomes and drug loaded polyion complex micelles is a promising strategy for the production of drug loaded nanocarriers with high stability, safety and bioavailability for oral drug delivery.

In our design, Pluronic F127 (PF127), FDA approved medicinal material with high ability of P-gp inhibition [12,13], was conjugated with positively charged polyethylenimine (PEI), the positively charged Pluronic F127-Polyethylenimine(PF127-PEI) was attached with negatively charged sodium cholate (CA) via electrostatic interaction. PTX loaded PF127-PEI/CA based polyion micelles were prepared and then encapsulated in the aqueous kernel of phospholipid based liposomes in order to improve the stability of PTX loaded polyion complex micelles in gastrointestinal fluid. It was hypothesized that encapsulation of polyion complex micelles into liposomes could protect them against the effect of variation of pH, especially the effect of pH in acidic gastric compartment. While in basic intestinal tract, PTX loaded PF127-PEI/CA based polyion micelles were stable because of the steric protection of hydrophilic chain PEO in PF127 molecule and the negatively charged CA which induced the electrostatic interaction with positively charged PF127-PEI. The drug loading capability, particle size, geometric properties, drug release profile, cell uptake, and the absorption of PTX in intestine were all evaluated to sufficiently assess the potential of this kind of hybrid liposomes as a kind of carrier for oral delivery of antitumor compound, PTX.

2. Materials and methods

2.1. Materials

Paclitaxel (PTX) was purchased from Chengdu Furunde Industrial Co. Ltd. (Chengdu, China). Soybean phosphatidylcholine (SPC, injection grade, PC > 95%) was supplied by Shanghai Aivait Medicine Co. Ltd. (China). Sodium cholate (CA), pyrene and rhodamine-123 (Rh-123) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Pentobarbital sodium was purchased from Guangzhou Chemical Reagent Co. Ltd. (Guangzhou, China). Verapamil and DMSO were purchased from Siyou Chemical Reagent Co. Ltd. (Tianjin, China). Pepsin and pancreatin were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents and buffer solution components were analytical grade preparation. Distilled and deionized water was used in all experiments.

2.2. The synthesis of copolymer PF127-PEI

PF127-PEI was synthesized according to the reported method with modification [14]. Purified Pluronic F127 (2.5 g, 0.2 mmol) was dissolved in dry tetrahydrofuran (THF) (30 ml), and then the solution was added into the THF solution of 1,1-carbonyldiimidazole (CDI, 48.62 mg, 0.30 mmol). The mixed system was stirred gently at room temperature for 6 h at nitrogen atmosphere to obtain CDI-activated Pluronic F127. The CDI activated Pluronic F127 was precipitated three times in ice-cold diethyl ether and dried under vacuum for 12 h. In order to obtain PF127-PEI, 1 ml of dichloromethane solution containing 100 mg of CDI-activated Pluronic F127 was added dropwise into 10 ml aqueous solution of PEI(0.04% w/v, pH = 9). The mixture was sonicated with a Branson sonifier 450 for 3 min and the whole system was evaporated with the method of rotary evaporation at 30 °C until the solution became clear to remove residual solvent. The solution was neutralized with hydrochloric acid and dialyzed (MWCO = 50,000) against aqueous ammonia solution (0.01%). The structure of synthesized PF127-PEI was confirmed with the method of Fourier transform infrared (FT-IR) and proton nuclear magnetic resonance (^{1}H NMR) analysis.

2.3. The preparation of drug-loaded polyion complex micelles

To prepare PTX-loaded PF127-PEI/CA polyion complex micelles, PTX (6 mg) and amphiphilic polyion complex copolymers (40 mg) comprised of different proportion of PF127-PEI and CA were dissolved in chloroform (4 ml), then the method of rotary evaporation was applied to remove the organic solvent and the film obtained was freeze-dried. The film was hydrated with 5 ml of 5 mM HEPES-buffered saline (HBS) and sonicated for 30 min. The resulting mixture was filtered through a 0.45-mm Nylon filter. The final samples were freeze dried and drug-loading content was determined.

2.4. The preparation of PTX loaded hybrid liposomes

PTX-loaded hybrid liposomes were prepared through incorporating PTX loaded PF127-PEI/CA polyion complex micelles into liposomes consisted of phospholipids with two different methods. In the first method, 5 ml of ethanol solution of phospholipids (6 mg/mL) was added dropwise to the aqueous solution of PTX loaded polyion complex micelles prepared in Section 2.3. After being sonicated for 30 min (100 W), the residual ethanol was removed through rotary evaporation [15]. The resulting liposomes were diluted with an equal volume of 5 mM HEPES-buffered saline (HBS) and then sonicated by a probe sonication for one or two 10 min cycles. The drug loaded liposomes were separated from the free drug by centrifuging at 10,000 rpm (7500 g) and liposomes were collected after removing the supernatant. The formed residue was reconstituted with 5 ml of distilled water followed by freeze drying. All freeze-dried liposomal powders were stored in a sealed glass vials at 4 °C for further analysis.

In the second method, PTX (6 mg), polyion complex copolymer comprised of PF127-PEI and CA (40 mg) and phospholipids were respectively dissolved in ethanol (10 ml) in a 50 ml flask and mixed sufficiently, then the ethanol was removed through rotary evaporation and the obtained film was hydrated with 5 ml distilled water [10]. The drug loaded liposomes were separated from the free drug by centrifuging at 10,000 rpm and nanoparticles were collected after removing the supernatant. The formed residue was reconstituted with 5 ml of distilled water followed by freeze drying. All freeze-dried liposomal powders were stored in a sealed glass vials at 4 °C for further analysis.