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Preparation, formula optimization and antitumor actions of mannitol coupling camptothecin nanoparticles

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ABSTRACT

The purpose of this work is to prepare a formulation using mannitol coupling Camptothecin (CPT) nanoparticles (CPT-NPs) to circumvent the difficult solubilization practice based on central composite experimental statistical design. CPT-NPs were prepared with a high-pressure homogenization technique method. The independent variables considered for the optimization of CPT-NPs were percentage of CPT in raw material (CPT and mannitol), concentration of CPT in working liquid, cycles numbers and homogenizer pressure for drug loading efficiency, particle size and polydispersity index. Analysis of variance (ANOVA) statistical test was used to assess the optimization. The optimized CPT-NPs showed an appropriate drug loading efficiency ($18.09 \pm 2.13\%$), a homogeneous particle size (165.33 ± 37.23 nm) and a low polydispersity index (0.29 ± 0.01). The CPT-NPs group show higher inhibition ratio (79.95%) of H22 tumor growth in vivo compared with TPT and CPT at the same dose. Changes in mice body weight demonstrate CPT-NPs have the lower toxicity. The results of biodistribution studies indicated the obviously superiority of CPT-NPs in increasing the accumulation of CPT within tumor. Overall, CPT-NPs under optimum conditions are considered to be potentially feasible to overcome formulation challenges for drug delivery.

1. Introduction

Camptothecin (CPT) is a natural alkaloid found in the bark of *Camptotheca acuminate* (Wall et al., 1966). CPT is attracted considerable attention worldwide, because of promising antitumor characteristics, which was discovered in the 1960s during screening of plant extracts for antitumor activity (Ma et al., 2012). CPT and its related analogs are effective anticancer agents that inhibit topoisomerase I to block DNA replication and RNA transcription (Li et al., 2004; Watanabe et al., 2008). While, CPT itself is not used clinically as an anticancer agent due to its poor water solubility and side effects (Zhu et al., 2012). The pH-dependent hydrolysis of CPT lactone form leads to the opening of the lactone ring and becomes the inactive carboxylate form (Selvi et al., 2008). CPT is commonly given as a sodium salt of the carboxylate form in clinics to overcome the poor solubility of the lactone form; this requires higher dose, which may lead to additional toxic reactions (Zhang et al., 2011). To improve solubility, investigators have successfully prepared formulations incorporating emulsions, micelles, liposomes, and nanoparticles (Garcia et al., 2010; Kim et al., 2011; Medina et al., 2011; Müller et al., 1996).

Mannitol (D-mannitol) is a six-carbon resistant sugar alcohol used in sweets and low-calorie foods. It is present in bacteria, yeast, fungi, algae, lichens, and many plants (Wisselink et al., 2002). Because of some features, mannitol is widely used in the pharmaceutical industry. Mannitol is a kind of water-soluble solid dispersions of carrier materials (SDS), and can improve solubility of the drug. Furthermore, mannitol contains six hydroxyls, and can easily form hydrogen bond with drug containing lone pair electrons in the atom. The hydrogen bonding capabilities of the sugar and amide group are new exploitable motifs (Moynihan et al., 2013).

Response surface methodology (RSM) is a collection of statistical and mathematical techniques that has been successfully used for developing, improving and optimizing biochemical processes (Chandrika and Fereidoon, 2005). When many factors and interactions affect desired response, RSM is an effective tool for optimizing the process (Luo, 2012). RSM is preferred as it can

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determine the effect of factors on characteristic properties, the best optimal conditions of process, and parameters interactions (Cai et al., 2007). The main advantage of RSM is to reduce the experimental runs required than be needed in a full factorial design, and it is already widely applied to optimize formulation design in pharmaceutics studies (Hatambeygi et al., 2011). Central composite design (CCD) is a popular form of RSM. CCD technique is a very useful tool that provides statistical models which help in understanding the interactions among the parameters that have been optimized (Nasirizadeh et al., 2012). This method is suitable for fitting a quadratic surface, and it helps to optimize the effective parameters with a minimum number of experiments and also to analyze the interaction between the parameters (Branchu et al., 1999).

In this study, we prepared mannitol coupling CPT nanoparticles based on central composite experimental statistical design and investigated their release curves, physical properties, and antitumor activities in vivo.

2. Materials and methods

2.1. Materials

Camptothecin (purity > 99%) was provided from Hisun Pharmaceutical Co. Ltd. (Zhengjiang, China). Mannitol (analytical grade) was purchased from Aladdin Regent (Shanghai, China). Topotecan (TPT, purity > 99%) was purchased from Tianyuan Technology Co. Ltd. (Chengdu, China). Dimethyl sulfoxide (DMSO, purity = 99.7%) was purchased from Aladdin Regent. Ethanol, acetonitrile and methanol were high-pressure liquid chromatography grade. The deionized water was pretreated with the Milli-Q plus system (Millipore, Bedford, MA).

2.2. Preparation of microCPT (mCPT)

The mCPT (micropowder mean particle size <1000 nm) were prepared by anti-solvent and supercritical technology. 16 mg unprocessed CPT was dissolved with 1 mL DMSO at 60 °C until the solution was clear yellow. 2 mL ethanol was then added dropwise to the CPT solution under constant stirring. At the end of equilibrium time, the dispersion was filtrated. The solid material was dried for 1 day in a vacuum drier at room temperature, and a yellow powder was obtained. The powder was processed to remove the DMSO and ethanol by supercritical CO_2 fluid extraction based on the method used in the early research. The important parameters in the supercritical antisolvent (SAS) technique: CO_2 flow speed of 13.3 mL/min, precipitation temperature of 35 °C, pressure of 20 MPa (Zhao et al., 2010)

2.3. Preparation of mannitol coupling CPT nanoparticles (CPT-NPs)

The CPT-NPs were prepared by a high-pressure homogenization technique. In brief, mCPT was dispersed in deionized water (pH 6). The dispersion liquid was added to high-pressure homogenizer. Then 2 mL aqueous solution of mannitol was added dropwise to the CPT liquid in the high-pressure homogenizer. After 3 cycles, the mixed solution was lyophilized to obtain dry white powder. Some parameters (i.e., the numbers of cycles) would be optimized in the next experiment content.

2.4. High-performance liquid chromatographic (HPLC) analysis of drug loading efficiency

To weaken the hydrogen bonding interaction, ultrasonic processing was developed to separate CPT and mannitol, and encapsulation efficiency was determined by using an ultraviolet spectrophotometer. Briefly, 5 mg CPT-NPs were dissolved with 1 mL DMSO at 60 °C until the solution was clear. Then the solution was ultrasonically processed. The liquid was diluted 1000-fold in DMSO. The solution centrifuged at 12,000 rpm for 10 min. The supernatant was then injected (20 μL) into HPLC system. The mobile phase consisted of acetonitrile and water (35:65, v/v); pH was adjusted to 5.5 by acetic acid at a flow rate of 1 mL/min. The column temperature was maintained at room temperature. All samples were detected in triplicate at a wavelength of 370 nm. The drug loading efficiency was calculated by the following expression:

$$\text{Drug loading efficiency}\ (\%) = \frac{\text{Weight of the drug in CPT} - \text{Nps}}{\text{Weight of the CPT} - \text{Nps}} \times 100\% \quad (1)$$

2.5. Particle size and polydispersity index analysis

The particle size and polydispersity index of CPT-NPs were detected by a dynamic light scattering analyzer (Brookhaven Instrument, USA). The freeze-dried samples of CPT-NPs were dispersed in deionized water and diluted to appropriate concentrations for examination. The samples were measured for a minimum of 45 s. Both particle size and polydispersity index measurements were determined in triplicate, results are reported as the average.

2.6. Response surface methods (RSM) experimental design

Initially, a 4-factor central composite design (CCD) with a reasonable starting point was utilized to define experimental factors and design space to explore (data not shown). The values for the responses drug loading efficiency, particle size and polydispersity index of CPT-NPs were analysed and the mathematical model for each response was generated in Table 1. In agreement with the experimental design, the response surface method normally approximates the correlation function as a full quadratic equation (Eq.) as follows:

$$Y = B_0 + \sum_{i=1}^{4} B_i X_i + \sum \sum_{i(j}^{4} B_{i,j} X_i X_j + \sum_{i=1}^{4} B_{i,j} X_i^2 + \epsilon \quad (2)$$

where Y were separately drug loading efficiency, particle size and polydispersity index of CPT-NPs, the X_i terms were the factors

Table 1
Levels of the parameters studied in the CCD statistical experiment.

Variable	Variable code	Coded variable level				
		−2	−1	0	+1	+2
Percentages of CPT in raw material (CPT and mannitol) (100%)	X_1	0.05	0.13	0.2	0.27	0.35
Concentration of CPT in working liquid (mg/mL)	X_2	0.1	0.3	0.5	0.7	0.9
Numbers of cycles	X_3	9	12	15	18	21
Pressure of the homogenizer (bar)	X_4	650	675	700	725	750