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Soluplus® based 9-nitrocamptothecin solid dispersion for peroral administration: Preparation, characterization, *in vitro* and *in vivo* evaluation

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ABSTRACT

Our study aimed to develop an amorphous 9-nitrocamptothecin solid dispersion (9-NC-SD) using polyvinyl caprolactam–polyvinyl acetate–polyethylene glycol graft copolymer (Soluplus®) for improving its oral bioavailability and antitumor efficacy *in vivo*. Freeze-dried 9-NC-SD with an optimized drug/polymer ratio at 1:15 (w/w) was characterized by powder X-ray diffraction, scanning electron microscopy and Fourier transform infrared spectroscopy. The amorphous form of 9-NC was obtained by freeze-drying and the aqueous solubility of 9-NC was increased to 1.42 mg/mL. Upon dilution, 9-NC-SD was proven to form micellar structures with an average size distribution around 58 nm ± 5 nm (PDI = 0.107 ± 0.016). Moreover, 9-NC-SD showed significantly increased intracellular uptake efficiency in Caco-2 cells compared to free 9-NC. Furthermore, the $AUC_{0-8\,h}$ of 9-NC-SD following oral administration showed a 2.68-fold increase in the lactone form of 9-NC compared to that of free 9-NC in Sprague-Dawley rats. The 9-NC-SD did not show obvious inflammatory responses and gastrointestinal toxicity following oral administration as demonstrated by the histological analysis of the rat intestinal sections. Thus, 9-NC-SD represents a promising approach for improving the solubility and oral bioavailability of drugs with poor solubility.

1. Introduction

9-nitrocamptothecin (9-NC), an analog of camptothecin (CPT), is a potent chemotherapeutic compound for the treatment of pancreatic cancer and other solid tumors (Haglof et al., 2006). As a second-generation topoisomerase-I inhibitor, pharmacological studies found that 9-NC displayed better antitumor activity than CPT and other CPT analogs in xenografted human tumors in nude mice (Pantazis et al., 1993). The antitumor efficacy of 9-NC is structure-specific: the pentacyclic structure of 9-NC with an α-hydroxy-δ-lactone moiety shows high antitumor activity (Fig. 1A) (Saha et al., 2013). However, under physiological conditions, 9-NC may undergo ring opening hydrolysis which leads to the formation of the inactive carboxylate form (Fig. 1B) (Gao et al., 2008). Thus, maintaining 9-NC in its active lactone form represents the primary goal for formulation development.

Despite the antitumor potency of 9-NC, the oral bioavailability of 9-NC remained extremely low mainly due to its poor water solubility (<5 μg/mL in distilled water at 25 °C) (Verschraegen et al., 1998), and low permeability (Sha and Fang, 2004), thus resulting in poor therapeutic efficacy and various adverse effects such as neutropenia, thrombocytopenia and gastrointestinal reactions (Venditto and Simanek, 2010). To improve the solubility and bioavailability of 9-NC, oil and glycol solutions (Burcham et al., 1997), self-emulsifying drug delivery systems (Lu et al., 2008) and liposomes (Knight et al., 1999) were explored during the past decade, which inevitably involved the usage of oils and surfactants such as Tween 80, Cremophor EL, which were proven to induce hypersensitive reactions in humans (Tong et al., 2012). Recently, a liposomal aerosol formulation has been successfully developed and already subjected to preclinical and clinical studies (Knight et al., 2000). However, the aerosol formulations must require special devices such as inhalers for administration which is less user-friendly and cost-effective compared to oral formulations (Smith and Parry-Billings, 2003). Thereby, solid dispersions, *i.e.*,

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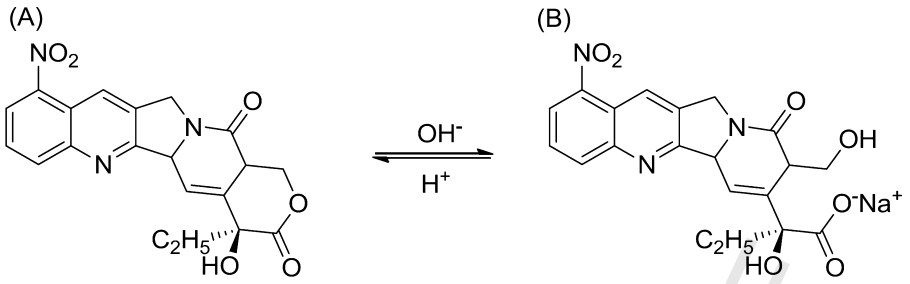

Fig. 1.

drug compounds that are dispersed amorphously in a hydrophilic matrix material, appear to be an attractive alternative for enhancing drug solubility and bioavailability (Chiou and Riegelman, 1971). In addition, solid dispersions can be further developed into dosage forms for oral administration such as tablets and capsules, which are highly acceptable in patients. Soluplus®, a polyvinyl caprolactam–polyvinyl acetate–polyethylene glycol (57/30/13) graft copolymer, has been extensively used in the fourth-generation of solid dispersions such as itraconazole (Zhang et al., 2013), carvedilol (Shamma and Basha, 2013), and CPT (Thakral et al., 2012). As a solubility enhancing excipient, Soluplus® carries multiple advantages such as minimum toxicity, low hygroscopicity and amphiphilicity. Compared with matrix materials such as Solutol® HS 15, Cremophor® RH 40 and Tween® 80 (Strickley, 2004), Soluplus® can serve either as a solubilizer to form micellar structures in the aqueous medium or as a matrix material for solid dispersions. Moreover, Soluplus® based solid dispersions were demonstrated to significantly improve the solubility or oral bioavailability of poorly water-soluble drugs (Kawabata et al., 2011).

In this study, the Soluplus®-based solid dispersion system has been applied for the first time to 9-NC. The present study aimed to develop and characterize the optimal formulation of Soluplus® based solid dispersions for 9-NC *via* freeze-drying, and to assess its oral bioavailability, antitumor activity and gastrointesetinal safety compared with free 9-NC suspensions and 9-NC/Soluplus® physical mixtures in animals. In addition, a preliminary study was conducted to explore the absorption mechanism of 9-NC-SD using Caco-2 cell monolayers in the current study.

2. Materials and methods

2.1. Materials

Soluplus® was kindly gifted by BASF SE (Ludwigshafen, Germany). 9-NC (purity >98.0%) was purchased from Chengdu Lanbei Plant & Chemical Technology Co., Ltd. (China). Dulbecco's modified eagle's medium (DMEM), fetal bovine serum (FBS), Hank 's balanced salt solution (HBSS), L-glutamine, penicillin–streptomycin, trypsin–EDTA and non-essential amino acids were obtained from Gibco Laboratory (Invitrogen Co., Grand Island, NY, USA). Transwell permeable membrane inserts (0.4 μm pore size, 1.12 cm^2 membrane area, polycarbonate) were purchased from Corning Incorporated Life Science (Lowell, MA, USA). All other chemical reagents were of HPLC grade.

2.2. Cell culture

Caco-2 cells were obtained from American Type Culture Collection (Manassas, CA, USA). Caco-2 cells (passages: 40–50) were cultured under the same condition as previously reported (Gao et al., 2011). For cellular uptake studies, cells were seeded in 12-well plates at a density of 50,000 cells/cm^2 and cultured for 14 days. For transport experiments, cells were seeded on transwell permeable membrane inserts at a density of 50,000 cells/cm^2 in 12-well plates and cultivated for 21 days. Monolayers with transepithelial electrical resistance (TEER) value higher than 600 Ω cm^2 were used in the transport assay.

2.3. Animals

Healthy male Sprague-Dawley rats were obtained from the Experimental Animal Center of Sichuan University (Chengdu, China). ICR mice were supplied by Chengdu Dossy Biological Technology Co., Ltd. (Chengdu, China). All animal experiments were approved by Sichuan University Animal Ethical Experimentation Committee (Chengdu, China), according to the requirements of the National Act on the use of experimental animals (People's Republic of China).

2.4. LC-MS/MS

A HPLC tandem triple quadrupole mass spectrometry (LC-MS/MS, 6410B, Agilent Technologies, Santa Clara, CA, USA) was used to analyze 9-NC. A diamonsil column (ODS, 50 × 4.6 mm, 1.8 μm) was used. The column was maintained at 30 °C. The mobile phase consisted of 0.02% formic acid and methanol (55:45, v/v). The flow rate was set at 0.4 mL/min. The injection volume was 3 μL. The quantitative analysis was performed using multiple reaction monitoring (MRM) mode. General mass spectrometer conditions were listed as follows: 9-NC transition (*m*/*z*), 394–350; fragmentor, 169 eV; collision energy, 24 eV; gas temperature, 350 °C; nebulizer pressure, 35 psi and spray voltage, 4000 V.

2.5. Sample preparation and solubility test

The 9-NC-SD was prepared by freeze-drying. Mixtures of 9-NC and Soluplus® with varying w/w ratios such as 1:5, 1:10, 1:15, 1:20 and 1:25 were dissolved in dimethylsulfoxide (DMSO). The solutions were frozen at −40 °C for 8 h, and then lyophilized for 24 h at −45 °C using Allegra X-22R freeze-dryer (Beckman Coulter, USA). Physcial mixtures of 9-NC and Soluplus® (9-NC-PM) of same w/w ratios were obtained by simple mixing in a mortar with pestle using geometric dilutions.

An excess amount of free 9-NC, 9-NC-PM and 9-NC-SD were placed in 5 mL of distilled water, and incubated in a shaking water bath at 37 ± 0.5 °C for 48 h. The supernatant was collected and filtered through a 0.45 μm membrane filter, and diluted appropriately with methanol. The concentration of 9-NC was then determined by LC-MS/MS.

2.6. Characterization of physicochemical properties

X-ray diffraction (X-RD) patterns of 9-NC, Soluplus®, 9-NC-PM, and 9-NC-SD were recorded by an X-ray diffractometer (X'Pert Pro MPD, The Netherlands Philips), using nickel-filtered Cu Kα

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