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Optimization and evaluation of Oridonin-loaded Soluplus®-Pluronic P105 mixed micelles for oral administration

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ABSTRACT

In this study, a new type of mixed micelles was developed using Soluplus® (SOL) and Pluronic® P105 (P105) for the encapsulation of Oridonin (ORN). Oridonin-loaded micelles (ORN-M) were simply prepared using solvent evaporation and characterized for particle size, particle morphology, encapsulation efficiency, and drug loading. In addition, the *in vitro* drug release behavior of ORN-M was assessed using the widely applied dialysis bag technique. The pharmacokinetic property of ORN was explored in rats after oral administration of ORN-M. Optimized ORN-M were of a small size (137.2 ± 1.65 nm) and spherical shape when the ratio of SOL:P105 was 3:1, with entrapment efficiency $90.48 \pm 1.85\%$ and drug loading $15.08 \pm 0.38\%$. Oral absorption capacity of ORN was greatly enhanced with a relative bioavailability of 210.55% in comparison to that of in-house suspensions, which suggests that ORN-M shows significantly improved bioavailability and drug absorption characteristics. Overall, the optimized SOL-P105 dual mixed micelles show great potential for use as oral drug carriers for cancer treatment.

1. Introduction

According to a World Health Organization report, cancer has been the leading cause of human mortality globally, with the total number of cancer patients predicted to double by 2050. Therefore, cancer is an outstanding public health problem worldwide, which needs to be solved urgently (Edwards et al., 2002). In addition to screening for new antitumor agents, which is a time-consuming, large-input, and high-risk process, there is an urgent need for research on new drug delivery systems.

While the oral route has been the most popular route of drug administration, the treatment efficacy of conventional oral dosage forms has been limited, as many of these Biopharmaceutical Classification System (BCS) Class II agents usually show low oral bioavailability mainly owing to poor solubility and dissolution rates (Fakes et al., 2009; Zhang et al., 2016; Ozdemir and Erkin, 2012). Formulation techniques, such as amorphous solid dispersion (Bochmann et al., 2016; Sun and Lee, 2015), β-cyclodextrin inclusion (Yao et al., 2014; Bourkaib et al., 2013), and nanosuspension (Ahuja et al., 2015; Gora et al., 2015) have shown great potential in enhancing drug dissolution and release. However, rapid dissolution does not mean well absorption, which leads to dissatisfaction of patients requiring drug formulations with high bioavailability and low dosage frequencies. Some attempts at improving drug absorption have involved the use of microspheres (Terada et al., 2016; Fan et al., 2016), liposomes (Kaminski et al., 2016; Eloy et al., 2016), multi-layered tablets (Mcconville et al., 2016; Park et al., 2011), and micelles (Figueroa-Ochoa et al., 2016; Zhang et al., 2015). Of these, mixed micelles, in particular, are becoming promising, tumor-preferential nano-carriers for oral delivery of poorly water-soluble drugs (Ponta and Bae, 2014).

Micelles are self-assembled nanoparticles formed by the organization of amphiphilic molecules into a hydrophobic core–hydrophilic shell structure (Moffitt et al., 1996; Li et al., 2014). The application of polymeric micelles for encapsulating hydrophobic drugs (Zhao et al., 2016a,b; Cheng et al., 2015) within the core with the outer shell providing the desired pharmacokinetic properties is becoming increasingly popular. In general, the dimension and

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shape of micelles can be controlled by varying the type and ratio of copolymers used. Particularly, the uniform size distribution and unique core-shell structure of mixed micelles lead to enhanced solubility, improved stability, sustained release, targeted distribution, and increased efficacy, while preventing drug deactivation (Hou et al., 2016; Chang et al., 2016). Thus, mixed micelles have been regarded as promising oral dosage forms for tumor chemotherapy.

Oridonin (ORN, Fig. 1) is a natural diterpenoid ($C_{20}H_{28}O_6$) isolated from *Rabdosia rubescens*, a universally applied herb in the clinical practice of traditional Chinese medicine. Some studies show that ORN has appreciable activity against various tumors (Wu et al., 2016; Ning et al., 2010; Liu et al., 2016). However, long-term and inappropriate drug administration can lead to drug resistance and low sensitivity to chemotherapy (Huang et al., 2005), which may be partly due to the efflux effect of P-glycoprotein (P-gp). Thus, the great potential for clinical application of ORN is severely limited by its insolubility and low bioavailability. Hence, developing a suitable pharmaceutical formulation of ORN is of great interest.

New techniques have been investigated to overcome these pharmaceutical problems, which involve in nanogels, nanoparticles, liposomes and nanosuspension for injection (Duan et al., 2011; Li et al., 2013; Wang et al., 2016; Lou et al., 2011). Although these injections provide controlled drug release, prolonged blood circulation and targeted tissue effect, clinic application was limited due to poor patient's compliance, such as severe pain and auxiliary magnetic, photodynamic, hyperthermia operation (Sanz et al., 2017; An et al., 2016; Kim et al., 2016). Hence, developing a suitable oral pharmaceutical formulation of ORN is of great interest. Among many oral preparation techniques, mixed micelles system is one of the most promising and effective carrier systems, which can significantly improve the solubility of hydrophobic drugs due to the core–shell structure (Chen et al., 2015).

In this study, nano-sized mixed micelles composed of Soluplus® (SOL) and Pluronic® P105 (P105) were formulated for the oral delivery of ORN. As a new polyvinyl caprolactam–polyvinyl acetate–polyethylene glycol graft copolymer, SOL possesses amphiphilic properties, solubilization capacities, and security attributes. Successful use of SOL to prepare sustained drug delivery systems is well-documented (Bernabeu et al., 2016; Dian et al., 2014; Yu et al., 2013). P105, a safe excipient with a greater proportion of poly (propylene oxide), facilitates the rapid formation of small, uniform micelles, omits an auxiliary homogenization process, increases drug loading, and inhibits P-gp-mediated drug efflux (Vyu et al., 1996; Husseini et al., 2009; Chen et al., 2013). However, micelles composed only of P105 are not perfectly stable because of the rather short chain length of poly (propylene oxide). As SOL possesses a very low critical micelle concentration value, which is helpful for improving the stability of the prepared micelles (Dian et al., 2014), combining SOL with P105 might overcome the deficiencies of P105 singular micelles mentioned above, thus offering a more effective and stable delivery system.

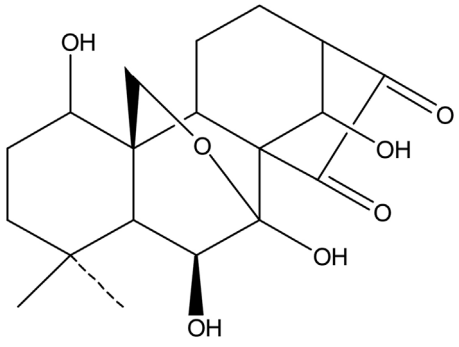

Fig. 1. Chemical structure of oridonin.

Thus, a novel mixed polymeric formulation was developed using SOL and P105 for improving water solubility, promoting intestinal absorption, and enhancing the antitumor capacity of ORN (Fig. 2). The physicochemical properties of ORN-loaded micelles (ORN-M), such as particle size, morphology, and *in vitro* release profile, were evaluated, and the *in vitro* cytotoxicity of ORN-M was investigated in comparison to with those of in-house suspensions. Furthermore, the antitumor activity and oral bioavailability were also assessed *in vivo* and the safety of ORN-M was evaluated through a gastrointestinal safety assay.

2. Materials and methods

2.1. Materials and reagents

ORN (purity > 98%) was procured from Jingzhu Technology Co., Ltd. (Nanjing, China). P105 was purchased from Sigma-Aldrich (St. Louis, MO, USA) and SOL from BASF Ltd. (Shanghai, China). Caco-2 TC7 cells were provided by Dr. Moniqué Rousset of INSERM U178 (Villejuit, France) and the human lung carcinoma cell line, PC-9, was received from Cellbio Inc. (Shanghai, China). Chromatographic grade methanol and acetonitrile were obtained from Tedia Company Inc., (USA). Double-distilled water was prepared for all biochemical assays. All other chemicals of analytical grade were from Sigma-Aldrich.

2.2. Animals

Healthy nude mice (22 ± 2 g) and male Sprague-Dawley (SD) rats (220 ± 20 g) were purchased from the Shanghai Laboratory Animal Center (Shanghai, China). All mice were housed under controlled conditions of 25 ± 0.5 °C temperature, 45 ± 5% humidity, and 12 h light/dark cycles for 1 week before the follow-up experiments. All animals were strictly handled according to the guidelines issued by the ethics committee of the Nanjing University of Chinese Medicine.

2.3. Preparation of ORN-M

ORN-M (1.0% w/v) were successfully prepared at 5.0% (w/v) polymer concentration by ethanol solvent evaporation method (Jin et al., 2014). SOL/P105 of different weight ratios and ORN were co-dissolved in absolute ethanol and the solution was vacuum-dried by using a RV8 rotary flash evaporator (IKA, Germany). The thin films thus obtained were mildly hydrated using a certain amount of deionized water at 25 °C for 30 min to form a uniform solution.

2.4. Characterization of ORN-M

2.4.1. Particle size, zeta potential, and morphology analysis

The hydrodynamic diameter and zeta potential of ORN-M were determined using dynamic light scattering using a Malvern Zetasizer Nano ZS instrument (Malvern Instruments, UK). Polydispersity index (PDI) was recorded with respect to time for assessing the particle size distribution.

For morphology analysis, samples were deposited on carbon-coated copper grids, followed by negative staining with a moderate amount of 2% phosphotungstic acid for 60 s. The stained grids were air-dried naturally and the morphology of ORN-M was observed