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High dispersed phyto-phospholipid complex/TPGS 1000 with mesoporous silica to enhance oral bioavailability of tanshinol

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ABSTRACT

Phenolic acids are widely distributed in the plant kingdom and possess a broad spectrum of pharmacological activity. However, low oral bioavailability restricts their application. In this study, a high dispersed phyto-phospholipid complex with mesoporous silica containing TPGS 1000 (TPC-SD) was fabricated using Tanshinol (Tan) as model drug. Phospholipid complex (PC) was employed to improve the *n*-octanol/water partition coefficient (log *P*) and apparent permeability coefficients (P_{app}) of Tan. Mesoporous silica was used to compensate for the negative effects of phospholipid on drug's dispersion and dissolution owing to its viscosity and poor water-solubility. TPGS 1000, a P-gp inhibitor, was used to block the efflux of Tan. Log *P* tests showed that the lipophilicity of Tan was significantly enhanced by Tanshinol phospholipid complex (TPC) formation. *In vitro* dissolution results showed that the cumulative dissolution percentage of Tan from TPC-SD was 4.33- fold of that from TPC in 120 min A Caco-2 permeability test confirmed that P_{app} (AP-BL) of TPC and TPC-SD increased approximately 2.22- and 3.53- fold compared to those of unformulated Tan, respectively ($p < 0.01$, $p < 0.01$). Pharmacokinetic studies demonstrated that the $AUC_{0-\infty}$ of TPC-SD was 2.23- and 1.47- fold compared with those of unformulated Tan and TPC, respectively ($p < 0.01$, $p < 0.01$). These results indicated that the high dispersed phyto- phospholipid complex/TPGS 1000 by mesoporous silica can be a promising drug delivery system to improve the oral bioavailability of free phenolic acids.

1. Introduction

Phenolic acids are widely distributed in the plant kingdom, especially in the Boraginaceae and Labiaceae families [1]. These components usually contain a aromatic group and an organic carboxylic acid function, such as the hydroxyl derivatives of benzoic and cinnamic acids [2]. Besides these ester, glycoside or amide forms, the unconjugated phenolic acids possess the free radical scavenging, metallic ion chelation and modulation of enzymatic activity [3]. They exhibit antiviral, anti-inflammatory, antitumor, anticoagulant, and cell protection activities [2]. However, their extremely low lipophilicity causes a poor permeability across intestinal epithelial cells and corresponding low oral bioavailability [4–6]. Furthermore, efflux of some free phenolic acids by P-glycoprotein (P-gp) further decreased the gastrointestinal membrane permeability [7,8]. To improve the oral bioavailability, many pharmaceutical approachesincluding nanoparticles, nanoemulsion and liposomes have been attempted before. But most researches are focused on nano-DDS, which are limited to apply by low entrapment efficiency, drug leakage, batch-to-batch irreproducibility and unfeasible industrialization, *etc.* Therefore, enhancement of the oral absorption of free phenolic acids remains an unsolved problem.

In recent years, the technique of complexing plant drugs with phospholipids has emerged as a challenging but successful method for improving the bioavailability and therapeutic efficacy of a number of poorly absorbed plant constituents [9]. Compared with other methods, it is relatively simple and safe. It incorporates phospholipid molecules containing phosphatidylcholine into complexes with standardized herbal extracts and/or specific active pharmaceutical plant ingredients to improve the *n*-octanol/water partition coefficient, membrane permeability and, hence, the systemic bioavailability of these drugs [10]. Although phospholipid complex (PC) may significantly increase membrane transport, its property of high viscosity and poor water-solubility

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makes the complex drug dispersion and dissolution rate low in aqueous media, including gastrointestinal fluid, which lead to negative effects on drug's absorption [11,12]. Several studies have shown that dissolution rates of active pharmaceutical ingredients were decreased in phyto- phospholipid complex compared to the crude drug. Khatik et al. [13] reported that the curcumin was completely released from crude drug within 6 h, while that from PC was only released by 49.43% within 24 h. Zhao et al. [14] showed that the release of tetrandrine decreased from 80% to 40% in 4 h after phospholipid complexation. Therefore, compensating method for the negative effects of phospholipid on drug's dispersion and dissolution is remained to be studied.

Recently, mesoporous silica strategies for drug dissolution enhancement have received increased attention [15]. Large surface area and pore volume make mesoporous silica materials be excellent candidates for effective drug loading and rapid release. Among those materials, Mobil Composition of Matter-48 (MCM-48) was typical representative as drug carriers to increase the dissolution rates of poorly water-soluble drugs [16].

As a major compound of phenolic acids in *Salvia miltiorrhiza Bunge*, Tanshinol (Tan, PubChem CID: 439435) exhibited a wide variety of pharmacological effects including anti-oxidant, anti-coagulant and anti-inflammatory activity, neuroprotective and cardioprotective effect, vasodilation, vascular reconstruction, and anxiolytic-like property [17–19]. As a BCS III class drug, its high hydrophilicity (log *P* from −0.58 to −2.48) with poor intestinal permeability ($P_{eff} < 1 \times 10^{-6}$ cm/s in caco-2 cell monolayer model) and hence low oral bioavailability (11.09%) impedes further clinical application [20–23]. Moreover, several studies have showed that Tan is a substrate for P-gp, which plays an important role in Tan when transporting the intestinal mucous membrane [22,24].

This work aimed to improve the oral bioavailability of free phenolic acids, using Tan as model drug, by fabricating high dispersed phyto-phospholipid complex with MCM-48, containing D-alpha-tocopheryl polyethylene glycol 1000 (TPGS 1000, PubChem CID: 71406) as a P-gp inhibitor [25]. Tan- phospholipid complex (TPC) and high dispersed TPC/TPGS 1000 with mesoporous silica (TPC-SD) were prepared using a solvent evaporation method and characterized by differential scanning calorimetry (DSC), Fourier-transformed infrared spectroscopy (FTIR), powder X-ray diffraction (PXRD), and scanning electron microscopy (SEM). The *n*-octanol/water partition coefficient (log *P*), *in vitro* dissolution, apparent permeability coefficients (P_{app}) and relative oral bioavailability of TPC-SD were also investigated.

2. Materials and methods

2.1. Materials

Tanshinol (raw material) was purchased from Nanjing JingZhu Bio-Technology Co., Ltd. (Nanjing, China), had a purity of mass fraction of more than 98.0%. A phospholipid, namely soybean lecithin containing 70–97% phosphatidylcholine, was purchased from Shanghai Tai-wei Pharmaceutical Co., Ltd. (Shanghai, China). TPGS 1000 was supplied by Sigma Co., Ltd. (St. Louis, MO, USA). Reference standards of Tanshinol and *p*-hydroxybenzoic acid (used as internal standard, *I.S.*, PubChem CID: 135) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China) with a purity of mass fraction of more than 98.0%. Vitamin C was obtained from Sinopharm Chemical Reagent Co., Ltd (Beijing, China). MCM-48 was procured from the Saint Chemical Materials Co. Ltd. (Shanghai, China). The structure parameters including mean particle size (PZ, μm), BET surface area (S_{BET}, m^2/g), pore volume (V_P, cm^3/g) were 43.39 μm, 1342 m^2/g and 1.01 cm^3/g. Methanol and formic acid (chromatographic grade) were purchased from Merck (Darmstadt, Germany). Purified water was prepared using a Milli-Q purification system (Millipore, Billerica, MA, USA). Other chemical reagents of analytical grade or better were obtained from Sinopharm Chemical Reagent Co., Ltd (Nanjing, China).

2.2. Animals

Male Sprague Dawley (SD) rats were obtained from the Experimental Animal Center of Nanjing University of Chinese Medicine (Nanjing, China). All procedures and experiments of animal study were approved by the Animal Care and Use Committee of Nanjing University of Chinese Medicine, and its approved protocol is "Scientific Protocol (2007) Number 16 of Nanjing University of Chinese Medicine".

2.3. Preparation of Tan- phospholipid complex (TPC) and high dispersed Tan- phospholipid complex/ TPGS 1000 with MCM-48 (TPC-SD)

TPC was prepared using a solvent evaporation method with drug to phospholipid at 1:2 wt ratios. Briefly, Tan and phospholipids were dissolved in anhydrous alcohol. The solution was refluxed at 40 °C for 2 h and evaporated under reduced pressure to give the solid product. The dried residues were gathered and further dried under vacuum at 40 °C for 12 h.

TPC-SD was prepared also with solvent evaporation technique. TPC and TPGS 1000 were dissolved into anhydrous alcohol at 1:3 wt ratio, and then a designated amount of MCM-48 (70% in TPC-SD, *w/w*) was added, stirred under room temperature for 30 min and then evaporated under reduced pressure at 40 °C to give the solid product. The dried residues were collected and further dried under vacuum at 40 °C for 12 h, and finally the TPC-SD was obtained.

Process optimization of TPC and TPC-SD including the weight ratios of drug to phospholipid in TPC, the weight ratio of Tan to TPGS in TPC-SD, and the amount of MCM-48 in TPC-SD were also studied and documented in the "Supplementary material".

2.4. Characterization of TPC and TPC-SD

Fourier transform infrared spectroscopy (FTIR). FTIR spectroscopy was used to investigate the possible interaction in the TPC and TPC-SD. The FTIR spectra of unformulated Tan, phospholipid, physical mixture of Tan and phospholipid (PM), TPC, TPGS 1000, MCM-48, the physical mixture of TPC, TPGS 1000 and MCM-48, and TPC-SD were obtained by the KBr method using FT-IR Spectrometer (Nexus 670, Thermo Nicolet, America). The spectra were recorded in range from 4000 to 400 cm^{-1} with a resolution of 2 cm^{-1}.

Differential scanning calorimetry (DSC). DSC study is a reliable method to screen drug-excipient compatibility and provides maximum information about the possible interactions. An interaction is concluded by elimination of endothermic peaks, appearance of new peaks, change in peak shape and its onset, peak temperature/melting point. The DSC thermograms of these above mentioned samples were recorded on a NETZSCH DSC-204 (Netzsch, Selb, Germany). Each sample (2–5 mg) was heated in aluminum pans at a scanning rate of 15 °C/min in an atmosphere of nitrogen gas from 30 to 300 °C.

Powder X-ray diffractometry (PXRD). PXRD was carried out to further investigate the crystalline state. The crystalline state of these above mentioned samples was determined using a powder X-ray diffractometer (D/Max-2500PC, Rigaku, Japan) by exposing samples to a Cu Kα radiation source at 40 kV and 25 mA. Data were collected over an angular range from 3° to 40° 2θ in continuous scan mode.

Scanning electron microscopy (SEM). The surface morphology of unformulated Tan, MCM-48 and TPC-SD was visualized by SEM (HITACHI S4800, Japan). The samples were placed on aluminum stubs using two-sided adhesive tape followed by coating with a thin film of gold for conduction. The images were obtained at an accelerated voltage of 1.0 KV. Surface morphological characteristics were viewed and photographed in the secondary electron mode.