European Journal of Pharmaceutics and Biopharmaceutics 92 (2015) 28–31

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research Paper

Loading amorphous Asarone in mesoporous silica SBA-15 through supercritical carbon dioxide technology to enhance dissolution and bioavailability

Zhengzan Zhang [a,b], Guilan Quan [a,b], Qiaoli Wu [a,b], Chan Zhou [a], Feng Li [a], Xuequn Bai [a,b], Ge Li [c], Xin Pan [a,b], Chuanbin Wu [a,b,*]

[a] School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou, China
[b] Research and Development Center of Pharmaceutical Engineering, Sun Yat-sen University, Guangzhou, China
[c] Guangzhou Neworld Pharmaceutical Ltd. Co., Guangzhou, China

ARTICLE INFO

Article history:
Received 22 October 2014
Revised 14 February 2015
Accepted in revised form 16 February 2015
Available online 23 February 2015

Keywords:
Mesoporous silica
Supercritical carbon dioxide
Hydrophobic molecules
Amorphous
Bioavailability
Dissolution

ABSTRACT

The aim of this study was to load amorphous hydrophobic drug into ordered mesoporous silica (SBA-15) by supercritical carbon dioxide technology in order to improve the dissolution and bioavailability of the drug. Asarone was selected as a model drug due to its lipophilic character and poor bioavailability. *In vitro* dissolution and *in vivo* bioavailability of the obtained Asarone-SBA-15 were significantly improved as compared to the micronized crystalline drug. This study offers an effective, safe, and environmentally benign means of solving the problems relating to the solubility and bioavailability of hydrophobic molecules.

1. Introduction

At present, about 40% of drugs in the development are poorly soluble in aqueous media, resulting in limited absorption and poor oral bioavailability [1]. Ordered mesoporous silica (SBA-15) was proposed as carrier for poorly water-soluble drugs to improve dissolution and bioavailability because it could not only increase the surface area dramatically but also entrap amorphous drugs in its nanometer pores [2]. However, organic solvents are most frequently used for drug dissolving and absorbing into the silica pores and their toxicity is a major concern.

It is known poorly soluble, small, non-polar or hydrophobic molecules can be easily solubilized in supercritical carbon dioxide (SC-CO_2), and the solubility can be modulated with the density of CO_2. Also, the drug molecules usually have high diffusivity in SC-CO_2 and can easily transfer into the nanopores of SBA-15. In addition, SC-CO_2 is a superior solvent because it is non-flammable, non-toxic, inexpensive, and has low and easily accessible critical temperature (31.1 °C) and pressure (7.38 MPa) [3]. Upon depressurization, CO_2 leaves the solid matrix without any residues.

α-Asarone (trans-1-propenyl-2,4,5-trimethoxyl-benzene) is one of the principal active ingredients of Acorus gramineus rhizome (AGR), which has been widely used as traditional medicine in China, Korea, India, and other Asian countries for several decades. Its pharmacological effects include hypolipidemic, neuroprotection, and antioxidant activity [4,5]. However, α-Asarone exhibits poor oral bioavailability due to its hydrophobicity [6].

Several studies have investigated drug loading in mesoporous silica using SC-CO_2 technology to enhance the *in vitro* dissolution [7,8]. In this study, hydrophobic Asarone was loaded into SBA-15 by SC-CO_2 technology and the Asarone-SBA-15 was evaluated both *in vitro* and *in vivo*.

2. Materials and methods

2.1. Materials

α-Asarone was obtained from Sanlian Pharm (Harbin, China). Triblock copolymer Pluronic® P123 (poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide), PEO_{20}–PPO_{70}–PEO_{20} (MW 5750), and tetraethyl orthosilicate were purchased from Sigma–Aldrich (St Louis, MO, USA). All other chemicals were of

* Corresponding author. Tel.: +86 02039943117; fax: +86 02039943115.
E-mail address: chuanbin_wu@126.com (C. Wu).

http://dx.doi.org/10.1016/j.ejpb.2015.02.018
0939-6411/

analytical grade and used as received. Water was purified by reverse osmosis.

2.2. Synthesis of SBA-15 mesoporous silica and preparation of micronized Asarone

SBA-15 mesoporous silica was synthesized according to the procedure originally reported by Zhao et al. [9]. Asarone was micronized by a jet mill (Model AO, Youte Instruments, Yixing, China) with compressed air at 0.7 MPa till the Sauter mean diameter of the powder was less than 10 μm. Particle size distribution was determined using a Malvern Mastersizer 2000 diffraction laser particle sizer (Malvern Instruments, Worcestershire, UK), equipped with a Scirocco dry powder unit (Malvern Instruments, Worcestershire, UK). The *in vitro* dissolution and *in vivo* bioavailability of the micronized Asarone were compared with those of Asarone-SBA-15 formulation.

2.3. Preparation of Asarone-SBA-15 with SC-CO_2

Fig. 1 schematically showed the experimental setup used for loading Asarone into SBA-15. SBA-15 and Asarone were both placed in the pressure vessel (100 mL), which was then pumped with CO_2 to pressure of 10 MPa and heated to 50 °C. Then the content in the vessel was equilibrated at the constant pressure and temperature for 60 min. Afterward, the vessel was depressurized by releasing CO_2, and Asarone-SBA-15 formulation was collected as dry powder, assayed by ultraviolet spectrophotometry (TU-1901, Beijing Purkinje General Instrument Co, Ltd, Beijing, China) at λ 258 nm to determine the Asarone content.

2.4. Characterization of Asarone-SBA-15

Powder X-ray diffraction (PXRD) analysis was performed with a Rigaku powder X-ray diffractometer (D-MAX 2200 VPC, Rigaku, Japan) using the Cu Kα radiation beam (λ 0.154 nm) operating at 40 kV and 40 mA. The scanning rate was 4°/min over a 2θ range of 5–40°.

Differential scanning calorimetry (DSC) study was carried out using Netzsch DSC instrument equipped with an intracooler (DSC 200 F3 Maia, Netzsch, Germany), which temperature and enthalpy scale were calibrated with Indium/Zinc standards. The samples were hermetically sealed in aluminum pans and heated at a constant rate of 10 °C/min over a temperature range of 30–80 °C. An inert atmosphere was maintained by nitrogen purging at a flow rate of 30 mL/min. The data were analyzed using Proteus analysis software.

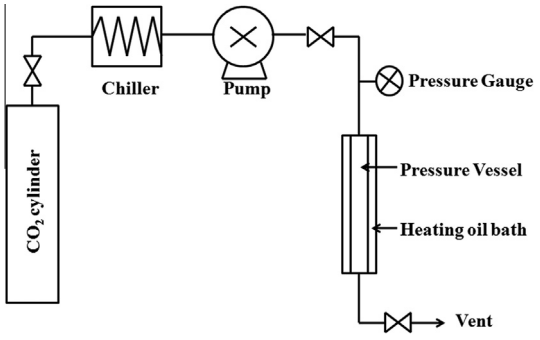

Fig. 1. Schematic graph of supercritical CO_2 apparatus for drug encapsulation in SBA-15.

2.5. In vitro dissolution of Asarone-SBA-15

Drug release from Asarone-SBA-15 and micronized Asarone powder was measured in 900 mL of 0.3% SDS solution as dissolution medium using the paddle method (USP apparatus 2) at the rotating paddle speed of 50 rpm. Dissolution samples (5 mL) were withdrawn at 5, 10, 15, 30, 45, and 60 min, and filtered through a 0.22 μm filter. Meanwhile, an equivalent amount of fresh medium was added to the dissolution vessel after each drawing to maintain a sink condition. The dissolution samples were analyzed by UV spectrophotometry at wavelength of 258 nm and the measurements were performed in triplicate.

2.6. In vivo study on Asarone-SBA-15

Six healthy female beagle dogs weighing 14 ± 1.0 kg were used in this randomized, crossover study, with a washout period of 1 week between the studies. Prior to the experiment, the dogs were fasted over night with free access to water. The treatment consisted of a single oral administration of Asarone-SBA-15 and micronized Asarone powder equivalent to a dose of 60 mg Asarone, together with 30 mL of water. At 0, 5,10, 15, 30, 45, 60, 90, 120, 150, and 180 min after administration, blood samples (5.0 mL) were withdrawn from the cephalic vein of the hind leg. Serum samples were obtained by centrifugation the blood samples and then stored at −20 °C till analysis. The animal study was approved by the Animal Ethics Committee of Sun Yat-sen University and performed in accordance with the National Institute of Health and Nutrition Guidelines for the Care and Use of Laboratory Animals.

2.7. Analysis of plasma Asarone concentration

A HPLC method was developed to determine the concentration of Asarone in plasma using a reversed phase HPLC equipped with a fluorescence detector (LC-20A, Shimadzu, Kyoto, Japan). Asarone and internal standard were separated by a C18 column (150 × 4.6 mm, 5 μm, GL science, Japan) guarded with a precolumn (GL science), and analyzed at the exciting wavelength of 255 nm and emission wavelength of 365 nm. The mobile phase consisted of methanol and water in a volume ratio of 65/35 and was pumped at a flow rate of 1.0 mL/min.

A 200 μL aliquot of plasma was spiked with methanol (20 μL) containing an internal standard (Celecoxib 50 μg/mL) and vortexed for 30 s. Then, ether (2 mL) was added as the extraction solvent, followed by vortexing for additional 3 min. After centrifugation at 10,000 rpm for 3 min, the supernatant was separated and the solvent was evaporated under vacuum at room temperature. The residue was reconstituted in mobile phase (200 μL) and then 10 μL of aliquot was injected for HPLC analysis.

2.8. Pharmacokinetic parameters

The area under the plasma concentration–time curve ($AUC_{(0\rightarrow\infty)}$) was calculated using the linear trapezoidal method. The peak plasma concentration (C_{max}) and the time to reach the peak plasma concentration (T_{max}) were determined from the experimental profile. The elimination rate constant (*Ke*) was estimated by regression analysis from the slope of the best fitting line, and the half-life ($t_{1/2}$) of the drug was calculated as value of 0.693/Ke.

2.9. Statistical analysis

The statistical analysis was performed using SPSS for Windows (version 17.0, SPSS Inc., Chicago, IL) and a Student's *t*-test. Data were reported as the mean with standard deviation,