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Preparation of osthole-polymer solid dispersions by hot-melt extrusion for dissolution and bioavailability enhancement

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ABSTRACT

The aim of this study was to investigate the potential of solid dispersion to improve the dissolution rate and bioavailability of osthole (Ost), a coumarin derivative with various pharmacological activities but with poor aqueous solubility. In present studies, the Ost solid dispersions were prepared with various polymers including Plasdone S-630, HPMC-E5, Eudragit EPO, and Soluplus by hot-melt extrusion method. *In vitro* characterizations were performed with differential scanning calorimetry (DSC), X-ray powder diffraction (XPRD), Fourier transform infrared (FT-IR) spectroscopy, and *in vitro* dissolution studies. In addition, *in vivo* pharmacokinetic studies of Ost solid dispersions were also conducted in rats after a single oral dose. In comparison to the untreated Ost coarse powder and the physical mixture with polymers, the solid dispersions prepared with Plasdone S-630 or HPMC-E5 (drug/polymer: 1:6) showed a significant enhancement of dissolution rate (~3-fold higher D30). In addition, such preparations exhibited a significantly decreased T_{max}, ~5-fold higher C_{max} and ~1.4-fold higher AUC when comparing with Ost coarse powder. In conclusion, solid dispersion prepared with appropriate polymer could serve as a promising formulation approach to enhance the dissolution rate and hence oral bioavailability of Ost.

1. Introduction

Osthole, a coumarin derivative, is the major bioactive constituent isolated from the fruit of *Cnidium monnieri* (L.) Cusson. It has been widely used for the treatment of skin disease and gynecopathy in Eastern Asians for hundreds of years. Modern pharmacological studies have shown that Ost exhibited various pharmacological effects (He et al., 2012; You et al., 2009), including anticancer (Xu et al., 2013), anti-osteoporosis (Ming et al., 2011), anti-inflammation (Wei et al., 2012), anti-apoptosis (Hou et al., 2009), and anti-allergic androgenic effects (Hsieh et al., 2004). Recently, Ost was reported to be effective for the treatment of alcoholic and milk-induced fatty liver disease (Du et al., 2011; Zhang et al., 2011). Although many pharmacological activities of Ost have been recognized, its aqueous solubility is relatively poor (intrinsic solubility estimated as 2 μg/ml). For such hydrophobic compound, poor solubility would resulted in a slow dissolution and hence low and erratic oral bioavailability, which may limit its further clinical application. So far, only few formulation strategies for improving dissolution of Ost have been investigated. For instance, Liu et al. significantly enhanced the dissolution rate of Ost by preparation of hydroxypropyl-β-cyclodetrin inclusion complexes (Liu et al., 2010). On the other hand, there is no report about *in vivo* pharmacokinetic performance of Ost in formulation development till now.

Various approaches are available to improve dissolution rate of poorly water-soluble drug, including the use of surfactants (Schott et al., 1982), inclusion complexation (Ammar et al., 1996), drug micronization into an amorphous form (Hancock and Zografi, 1997) and solid dispersion (Chiou and Riegelman, 1971). In the solid dispersion, the drug may be dispersed or solubilized within a polymeric carrier at molecular levels or in the amorphous state, and provide a large surface which significantly enhances the dissolution process. The improvement in dissolution is mainly attributed to the reduction in particle size, increase in surface area and reduction in crystallinity. Furthermore, no energy is required to break up the crystal lattice of a drug during the dissolution process, and drug solubility and wettability may be improved by surrounding hydrophilic polymers used in solid dispersions (Shinde et al., 2008). In comparison with traditional methods for preparation of solid dispersions, hot melt extrusion (HME), as a promising novel technology for improving the bioavailability of water-insoluble drugs, presents many advantages for pharmaceutical applications. It can be used as a continuous process with the absence of organic

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solvents and subsequent drying steps, which makes scaling-up easier (Djuris et al., 2013; Sarode et al., 2012). In addition, intense blending and agitation during process prevent the aggregation of drug particles suspending in the molten polymer, leading to a more homogeneous dispersion of fine particles (Feng et al., 2012). However, not all the thermal plastic polymer carriers are compatible with the drugs and suitable carriers as well we drug/polymer ratio for a specific drug need to be optimized.

In the present studies, we attempted to improve dissolution and bioavailability of Ost by preparation of solid dispersions with HME technique. Hydrophilic polymers with different glass transition temperatures and backbones will be used to prepare solid dispersions. Differential scanning calorimetry (DSC), X-ray powder diffraction (XRPD), Fourier transform infrared (FT-IR) spectroscopy, and dissolution studies will be conducted to compare the *in vitro* characterization and performance of prepared solid dispersions with coarse Ost power and physical mixtures of Ost and polymers. Further pharmacokinetics of Ost in rats was investigated to evaluate the *in vivo* performance of prepared solid dispersions.

2. Materials and methods

2.1. Materials

Ost (purity >98%; Fig. 1A) was purchased from Xi'an Tianben Bio-engineering Co. Ltd. (Xi'an, China). Ost standard and internal standard (IS) imperatorin (Fig. 1B) were obtained from the National Institute for the Control of Pharmaceuticals and Biological Products (Beijing, China). Eudragit EPO and Plasdone S-630 were kindly gifted from Evonic Degussa Corporation (Piscataway, NJ, USA) and ISP Technologies Inc. (Wayne, NJ, USA), respectively. Polyvinyl caprolactam–polyvinyl acetate–polyethylene glycol graft copolymer (Soluplus, BASF, Germany) and Hypromellose-E5 (HPMC) (Colorcon, Germany) were kindly donated from the manufactures. Lactose and Glucose were provided by GlaxoSmithKline (Ware, UK).

2.2. Solubility parameter calculations

Solubility parameter (δ) for Ost was performed by the group contribution method using molecular modeling pro software (ChemSW, Fairfield, CA, USA). The solubility parameters for the polymers (*i.e.* Eudragit EPO, Plasdone S-630, HPMC, Solupus, Lactose and Glucose) were taken from the literatures (Chokshi et al., 2005; Djuris et al., 2013; Forster et al., 2001; Sathigari et al., 2012) and matched to Ost by observing the relative difference in total, $\Delta\delta$. The differences ($\Delta\delta$) between the δ values of the drugs and the polymers were also determined.

2.3. Preparation of hot-melt extrudates

Composites of drug and polymer at different ratios of 1:3, 1:6 and 1:9 were applied to prepare solid dispersions by the Haake Minilab twin-screw extruder with counter rotating screws at 35 rpm. The temperatures for processing were selected according to the glass transition temperature (T_g) of the polymers and melting point of the drug. Due to the different physicochemical characteristics of the applied carriers, the temperature setting was varied to obtain a semi-solid, transparent strand for each formulation suitable for down-processing. In the present investigation, about 120, 165, 85 and 105 °C was applied for Plasdone S-630, HPMC, Eudragit EPO and Soluplus to prepare polymer–drug hot-melt extrudates, respectively. The homogeneous hot-melt extrudates were collected and allowed to cool down to room temperature for at least 24 h, and then pulverized to pass through a 60-mesh screen. The obtained powder of Ost solid dispersions was stored in a dryer at room temperature for further *in vitro* physicochemical characterization and *in vivo* pharmacokinetic evaluation.

2.4. Physicochemical characterization of solid dispersions

2.4.1. Drug content

Drug content in the prepared solid dispersions were analyzed by a validated HPLC method. The HPLC system consists of a solvent management system (quaternary gradient mode), auto injector, column oven and a 4 channel in line degasser, a sample management system and a 2998 PDA detector (Waters Corporation, USA). Chromatographic separation was performed on a reversed-phase C_{18} column (4.6 × 250 mm, 5 μm; Waters, USA) with an isocratic elution of methanol–water (80:20, *v/v*) at a flow rate of 1 mL/min. Absorbance was monitored at 320 nm. The column was maintained at 30 °C and the injection volume was 10 μL. Data acquisition was performed by the Empower 3 software.

2.4.2. DSC studies

To examine the effect of different polymers on the status of crystallinity of Ost in the prepared solid dispersions, pure drug Ost, each polymer, physical mixture of Ost and polymer, as well as prepared solid dispersions were investigated using DSC-204 instrument (Netzsch, Germany). Each sample was sealed in aluminum hermetic pans and heated from 40 °C to 200 °C at a heating rate of 10 °C/min in an atmosphere of nitrogen. Data analysis was performed using NETZSCH-Proteus software.

2.4.3. X-ray powder diffraction (XRPD)

To determine the physical state of pure drug, XRPD of the prepared solid dispersions and their binary physical mixtures were recorded using D/max 2500 PC XRD analyzer (Rigaku Corporation, Tokyo, Japan) with a Cu-Kα radiation source. Samples were gently placed in an aluminum holder. The tube voltage and amperage were set at 40 kV and 100 mA, respectively. Measurement was conducted in the 2θ range from 3° to 60° using a 0.02° step size and 1°/min scan speed.

2.4.4. FTIR studies

The FTIR spectra of the pure drug Ost, each used polymer, physical mixture of Ost and polymer, as well as the prepared solid dispersions were investigated by Bruker-MPA FTIR system (BRUKKER, Switzerland). The samples were mixed with KBr and pellets were prepared in the FTIR holder. Spectra were performed in the transmission mode in the region 4000 to 400 cm^{-1} using the resolution 2 cm^{-1}.

2.4.5. Dissolution testing

The dissolution studies of the coarse powder of pure drug Ost, physical mixture (PM) of Ost and polymer, as well as the prepared solid dispersions (each sample containing 4 mg of Ost) were performed using dissolution test method 2 as described in the Chinese Pharmacopeia 2010. The dissolution medium was 900 mL of pH 4.5 acetate buffer with 0.05% Tween 80. The temperature of dissolution medium was set at 37 °C and the paddle rotation speed was adjusted to 50 rpm. Samples (10 ml) were withdrawn at 5, 10, 20, 30, 45, 60, 90 and 120 min, filtered through a 0.45-μm Millipore filter, and analyzed by the HPLC method described above.

2.5. Pharmacokinetic study

2.5.1. Animals

Male Sprague-Dawley rats (180–220 g) were obtained from the Laboratory Animal Center of Nanjing Medical University (Nanjing, China). The rats were housed in an environmentally controlled breeding room (temperature maintained at 24 ± 2 °C and with a