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Improved oral bioavailability of probucol by dry media-milling

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ABSTRACT

The polymer/probucol co-milled mixtures were prepared to improve drug dissolution rate and oral bioavailability. Probucol, a BCS II drug, was co-milled together with Copovidone (Kollidon VA64, VA64), Soluplus, or MCC using the dry media-milling process with planetary ball-milling equipment. The properties of the milled mixtures including morphology, crystal form, vitro drug dissolution and in vivo oral bioavailability in rats were evaluated. Probucol existed as an amorphous in the matrix of the co-milled mixtures containing VA64, which helped to enhance drug dissolution. The ternary mixture composed of VA64, RH40, and probucol showed increased dissolution rates in both sink and non-sink conditions. It also had a higher oral bioavailability compared to the reference formulation. Dry-media milling of binary or ternary mixtures composed of drug, polymer and surfactant possibly have wide applications to improve dissolution rate and oral bioavailability of water-insoluble drugs.

1. Introduction

The number of active pharmaceutical ingredients (API) of biopharmaceutics classification system II (BCS II) is increasing. However, their oral availability is generally limited due to the low aqueous solubility. Improving the solubility/dissolution of poorly water-soluble compounds is a challenging task in the development of pharmaceutical preparations containing BCS II drug for oral delivery [1]. Various strategies have been introduced to improve the dissolution of poorly water-soluble drugs, such as particle size reduction (e.g. dry or wet milling), pro-drugs, cyclodextrin inclusion, self-emulsification, etc. [2–5]. Although several methods have been reported to improve dissolution and oral bioavailability of probucol [6–9], it is valuable to develop a simple, easily scale-up, solvent-free technology with low heat.

Milling is an important process to reduce particle size mechanically in chemistry, mineral processing, materials and pharmaceutical industries. The co-milling approach is more versatile in contrast to melting or solvent-evaporation methods. Melting is a temperature-dependent process. Many substances undergo thermal decomposition and impurities are increased at high temperature. For solvent-evaporation methods, residual solvents remaining in final product may create potential toxicity issues. Therefore, embedding a poorly water-soluble drug as a solid dispersion in a hydrophilic carrier by mild co-milling is a promising strategy for enhancing the dissolution of water-insoluble drugs. Several pharmaceutical products based on micronized or nanocrystal/nanosuspension techniques via milling method, have been successfully marketed [10]. While the wet-milling technique needs post-processing in production of nanosuspensions, the most important advantages of dry media-milling method are no solidification, good chemical stability, and easy processing of API into suitable dosage forms although agglomeration and temperature increase may occur.

Probucol is a potent anti-oxidative drug for the prevention and treatment of atherosclerotic cardiovascular diseases and xanthoma. It has also been used as a lipid-lowering drug before. Although Western countries withdraw it because of the reduction in serum high density lipoprotein cholesterol (HDL-C), recent animal and clinical studies reported favorable effects of probucol on atherosclerotic cardiovascular diseases. Its mechanisms of action have been explained at the molecular level. Although probucol is known to prolong QT interval on an electrocardiogram, no fatal ventricular arrhythmias have been reported even in positive studies. Potent anti-oxidative effect of probucol and enhanced reverse cholesterol transport (RCT) may explain its strong anti-atherosclerotic effects despite serum HDL-C reduction. Probucol has pleiotropic and beneficial therapeutic effects on cardiovascular system. Especially, it was one of the few drugs to prevent restenosis after percutaneous transluminal coronary (PTCA). Although statins are effective for lowering low density lipoprotein cholesterol (LDL-C) and reducing coronary heart disease risk, probucol should be considered as an option in case statins are not effective [11]. Currently, probucol is still widely used in some Asian countries, including China, Japan and Korea. The use of probucol, with its long history of safe use and established effectiveness, needs to be re-evaluated for its ability to

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reduce coronary heart disease risk and other lipid-lowering therapy. Western countries might have abandoned probucol too soon [11,12].

As a typical BCS II drug, the effectiveness of probucol (oral bioavailability < 10%) is greatly limited due to its extremely low water solubility (5 ng/mL) [13]. Therefore, effective methods are needed in order to improve its dissolution and oral bioavailability. In this study, polymer/probucol co-milled mixture with improved dissolution was successfully prepared by dry-media co-milling of probucol and pharmaceutical polymer(s) (MCC, VA64 or Soluplus). The physicochemical properties and oral adsorption of the co-milled mixture were evaluated as well.

2. Materials and methods

2.1. Materials

Probucol was purchased from Wuyi Cihang Pharmaceutical Co. Ltd. (Hebei, China). Kollidon® VA64 (Vinylpyrrolidone/vinyl acetate = 60/40, VA64), Soluplus® (Polyvinylcaprolactam-polyvinyl acetate-polyethylene glycol graft copolymer), and Cremophor®RH40 (RH40) were kindly donated by BASF Company Ltd. (Germany). Microcrystalline cellulose (MCC) was purchased from Changwei Pharmaceutical Company Ltd. (Shanghai, China). All other reagents used were of analytical or chromatographic grade.

2.2. Dry media-milling

Ball milling was performed with a PM0.4L high energy planetary mill (Chishun, China) equipped with 200 cm^3 zirconium oxide milling bowl containing 150 g beads (Ø = 0.5 mm) at room temperature and 1000 r/min. The mixtures of probucol and polymers were placed in the milling bowl at a beads/sample weight ratio of 30:1. To avoid temperature increase of the sample, pause periods of 10 min were set after every 30 min of milling. The resulted powders were sieved through a 100 mesh screen and stored at room temperature. The formulation and milling time of the sample were listed in Table 1 (F1–F3: unary milling formulations; F4–F6: binary co-milling formulations; F7–F9: ternary co-milling formulations).

2.3. Optical microscopic and scanning electron microscopy (SEM) observation

The milled mixtures were observed with a BA300POL microscope (Motic, China). Morphology of Samples sputtered with gold, were observed using S-3400 SEM (Hitachi, Japan) at 5.0 KV electron acceleration voltage from different random microscopic fields. The number of particle and particle size distribution was measured using the attached software from at least five representative regions.

2.4. Differential scanning calorimetry (DSC) analysis

DSC analysis was carried out using DSC-1 system (Mettler Toledo, Switzerland) in the temperature range of 30 °C–200 °C at a heating rate of 10 °C/min in a dynamic nitrogen atmosphere. Approximately 5 mg of sample was sealed in an aluminum pan and an empty sealed pan was used as the reference.

Table 1
Formulations of probucol and polymer/probucol mixtures.

	Ingredients, g					Effective milling time, h
	Probucol	MCC	Soluplus	VA64	RH40	
F1	5	–	–	–	–	2
F2	5	–	–	–	–	4
F3	5	–	–	–	–	6
F4	0.75	4.25	–	–	–	1
F5	0.75	–	4.25	–	–	1
F6	0.75	–	–	4.25	–	1
F7	0.75	–	–	3.6125	0.6375	1
F8	0.25	–	–	4.0375	0.7125	1
F9	1.5			2.975	0.525	1

2.5. In vitro drug release

The in vitro release test was performed using the paddle method as described in *Chinese Pharmacopoeia* (*2015*) using a ZRS-8G Dissolution Apparatus (Tianda Tianfa Technology Co. Ltd., China). In this study, 900 mL SDS (2% w/w) solutions (sink condition) or purified water (non-sink condition) at 37.0 ± 0.5 °C were used as the dissolution media respectively, and the rotation speed was set at 50 r/min. The milled samples equivalent to 3 mg or 15 mg probucol were added to the dissolution medium respectively, at pre-determined time intervals. The dissolution sample (5.0 mL) was withdrawn from the beakers and replaced with the equal volume of fresh medium. The dissolution samples were filtered through a membrane filter of 0.45 μm pore size (Millipore, USA) and the content of probucol was then assayed by LC-10AD high performance liquid chromatography (HPLC) (SHIMADZU, Tokyo, Japan) equipped with a Dikma® ODS C18 chromatography column (200 mm × 4.6 mm, 5 μm) and a UV–vis detector (242 nm). The mobile phase consisted of methanol and distilled water (100:3, v/v) and was pumped at a flow rate of 1.0 mL/min. Validation of the assay method showed good linearity in the concentration range of 0.2 μg/mL to 10.0 μg/mL ($A = 139911C + 5165.65$, $R^2 = 0.9995$) and precision (RSD < 2.0%).

2.6. In vivo pharmacodynamics study

Male Sprague-Dawley rats 190 g ± 20 g were obtained from the Laboratory Animal Center of Shenyang pharmaceutical University (Shenyang, China). The rats were fasted overnight before experiments. Rats were randomly divided into two groups, and orally administered with VA64/RH40/Probucol ternary co-milled mixture (test formulation) or milled powder of commercially available probucol tablet (reference formulation) at a dose of 250 mg probucol/kg body weight separately. Blood samples were collected into heparinized tubes from the suborbital vein at the following time points: predose, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h 12 h, 24 h, and 36 h. Plasma was separated by centrifugation at 5000 rpm for 10 min and then stored at −20 °C until determination. All procedures were performed according to the guidelines and approval of the Institutional Animal Experimentation Ethics Committee of Shenyang Pharmaceutical University.

For analysis, 200 μL internal standard (2.0 μg/mL amiodarone in methanol) was spiked to 200 μL of plasma sample, mixed vigorously for 1 min, and then 200 μL methanol was added and vortexed. 1 mL of *n*-hexane was added and vortexed for another 3 min. After centrifugation at 5000 r/min for 10 min, the organic phase was transferred to another tube and evaporated to dryness at 40 °C. The residue was reconstituted in 100 μL mobile phase, 20 μL of the solution was injected into the HPLC system. Probucol was assayed by LC-10AD HPLC (SHIMADZU, Tokyo, Japan) equipped with a Thermo C18 column (250 mm × 4.6 mm, 5 μm) and a UV–vis detector (242 nm). The mobile phase consisted of acetonitrile, distilled water and triethylamine (93:7:0.01, v/v/v) was pumped at a flow rate of 1.0 mL/min.

Maximum concentration (C_{max}) and maximum times (T_{max}) were obtained directly from the concentration versus time data. The other pharmacokinetic parameters ($AUC_{0-72\ h}$ and $t_{1/2}$) were analyzed by DAS 2.1.1 software (supplied by Chinese Pharmacological Society).

3. Results

3.1. Morphology and physicochemical properties

The microscopic and SEM images of milled samples were shown in Fig. 1. With the increase of milling time, the particle size of probucol