European Journal of Pharmaceutics and Biopharmaceutics 115 (2017) 65–72

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research paper

Enhanced pulmonary absorption of poorly soluble itraconazole by micronized cocrystal dry powder formulations

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ARTICLE INFO

Article history:
Received 4 October 2016
Revised 27 January 2017
Accepted in revised form 16 February 2017
Available online 20 February 2017

Chemical compounds studied in this article:
Itraconazole (PubChem CID: 3793)

Keywords:
Cocrystals
Crystal engineering
Amorphous
Dry powder formulation
Pulmonary absorption
Dissolution rate
Micronized particles

ABSTRACT

Micronized cocrystal powders and amorphous spray-dried formulations were prepared and evaluated *in vivo* and *in vitro* as pulmonary absorption enhancement formulations of poorly soluble itraconazole (ITZ). ITZ cocrystals with succinic acid (SA) or l-tartaric acid (TA) with a particle size diameter of <2 μm were successfully micronized using the jet-milling system. The cocrystal crystalline morphologies observed using scanning electron microscopy (SEM) suggested particle shapes that differed from those of the crystalline or spray-dried amorphous ITZ. The micronized ITZ cocrystal powders showed better intrinsic dissolution rate (IDR) and pulmonary absorption profile in rats than that of the amorphous spray-dried formulation and crystalline ITZ with comparable particle sizes. Specifically, in rat pharmacokinetic studies following pulmonary administration, micronized ITZ-SA and ITZ-TA cocrystals showed area under the curve from 0 to 8 h (AUC_{0-8h}) values approximately 24- and 19-fold higher than those of the crystalline ITZ and 2.0- and 1.6-fold higher than the spray-dried ITZ amorphous values, respectively. The amorphous formulation appeared physically instable during the studies due to rapid crystallization of ITZ, which was its disadvantage compared to the crystalline formulations. Therefore, this study demonstrated that micronized cocrystals are promising formulations for enhancing the pulmonary absorption of poorly soluble compounds.

1. Introduction

Pulmonary drug administration is a promising alternative route of drug delivery and absorption, based on its advantages of enabling systemic drug delivery and topical disease treatment. The avoidance of the first-pass metabolism is a key benefit of an alternative systemic administration route that can overcome the frequent poor bioavailability (BA) of the oral route. Compared to the nasal and buccal/sublingual cavities, the lungs have a huge available surface area (>100 m^2), good epithelial permeability, and modest metabolic capacity. Therefore, the pulmonary route has attracted considerable attention for numerous potential applications in systemic drug delivery [1].

In formulation research pertaining to pulmonary drug administration, the dry powder inhalation (DPI) formulation is widely used because of its advantages, namely ease of handling, superior chemical stability than that of solution formulations, and ease of using commercially available inhaler devices [2]. The extent of pulmonary absorption can be limited by the poor physicochemical properties of the drug, such as solubility, dissolution rate, wettability, and membrane permeability. Therefore, DPI formulation technologies that improve these properties and enhance the systemic delivery or topical activity of poorly absorbed drugs are urgently required [3,4]. In particular, the discovery of solubilization techniques for poorly soluble drugs is a major challenge in the development of inhalation products because of the strict limitation on

Abbreviations: AUC, area under the curve; BA, bioavailability; CCF, cocrystal former; DPI, dry powder inhalation; DPPC, dipalmitoyl phosphatidylcholine; DSC, differential scanning calorimetry; FA, fumaric acid; HPLC, high-performance liquid chromatography; HPMC-P, hydroxypropyl methylcellulose phthalate; IDR, intrinsic dissolution rate; ITZ, itraconazole; IV, intravenous; MA, l-malic acid; MRT, mean residence time; PK, pharmacokinetic; PS80, polysorbate80; PSA, particle size analysis; PTFE, polytetrafluoroethylene; PXRD, powder X-ray diffractometry; SA, succinic acid; SD, solid dispersion; SEM, scanning electron microscopy; TA, l-tartaric acid.

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http://dx.doi.org/10.1016/j.ejpb.2017.02.013
0939-6411/

administrable doses in DPI treatment [1,5]. Additionally, from a safety viewpoint, dry powders are required to rapidly dissolve in the lungs because persistent presence and accumulation of undissolved solids can induce lung irritation and topical side effects [6,7].

Some formulation strategies that enhance pulmonary absorption, such as nano-sized particles and amorphous solid dispersion (SD), have been proposed recently [3,4,8]. Duret et al. reported that following pulmonary administration to mice, the systemic absorption and local concentration of the poorly soluble itraconazole (ITZ), an azole antifungal, in the lungs increased more significantly with an amorphous formulation than with a crystalline powder [9]. Hence, amorphous formulations are a promising strategy for improving the pulmonary absorption of poorly soluble drugs through its solubilization effect as same in the case of oral administration. However, from the pharmaceutical development perspective, it should be essential to pay close attention to the physical and chemical stability issues. Amorphous formulations have a high energy state and could therefore have a higher risk of physical and chemical instability than crystalline substances [10,11]. To overcome the instability of amorphous compounds, polymeric stabilizers are well known to be used in amorphous SD formulations [12]. Engers et al. demonstrated that an ITZ amorphous SD formulation containing hydroxypropyl methylcellulose phthalate (HPMC-P) polymer significantly improved the oral BA in dogs [13]. Meanwhile, in the case of pulmonary administration, it is considered that non-biodegradable and high-molecular-weight materials should not be continuously introduced into the lungs that would cause the lung functional damage because of the accumulation of materials in the alveoli [14]. Cocrystallization is another technique for improving ITZ's solubility and dissolution rate, which is known to form cocrystals with various aliphatic dicarboxylic acids [15,16]. The expected advantages of cocrystals over amorphous formulations are its acceptable stability in pharmaceutical development and greater safety of cocrystal formers (CCFs) than that of the polymers in amorphous SD in the lungs. This is because there are many compound choices for CCFs, which are considered safe for the lungs. In cocrystal screening, hundreds of CCFs candidates that can be selected from counter ions generally used in salts, pharmaceutically accepted excipients, and generally recognized as safe (GRAS) substances database approved by the U.S. Food and Drug Administration (FDA) as food additives [17]. Despite the great potential of cocrystals, no studies have attempted to demonstrate their advantages in pulmonary drug delivery. Although it was only reported that the aerosolization performance of DPI powders was modified by cocrystallization of theophylline [18], *in vitro* dissolution or *in vivo* pulmonary absorption performance has not been evaluated to date. In this study, we developed a micronized cocrystal powder preparation to demonstrate its applicability for inhalation drug delivery by using ITZ as a model poorly soluble drug, so as to propose an additional approach for pulmonary absorption enhancement.

2. Materials and methods

2.1. Materials

ITZ (≥98.0% purity) was purchased from Tokyo Chemical Industry, Ltd., (Tokyo, Japan). The CCFs, SA, l-malic acid (MA), fumaric acid (FA), and TA were purchased from Wako Pure Chemical Industries, Ltd., (Osaka, Japan). All organic solvents used to prepare the cocrystals were purchased from Wako Pure Chemical Industries, Ltd. D-mannitol for the dry powder formulation was purchased from Roquette Pharma Corporation (Lestrem, France) while dipalmitoyl phosphatidylcholine (DPPC) and polysorbate 80 (PS80) for the dissolution medium were purchased from NOF Corporation (Tokyo, Japan).

2.2. Preparation methods

2.2.1. Preparation of ITZ cocrystals and amorphous

ITZ cocrystals with four aliphatic dicarboxylic acids, SA, MA, FA, and TA, were prepared using the anti-solvent crystallization method according to a previously reported procedure [19]. Specifically, 500 mg of ITZ and approximately 200 mg of each dicarboxylic acid were physically mixed and dissolved in 20 mL of tetrahydrofuran (THF) at 50 °C. The solution was filtered through a 0.22-μm membrane filter (Millex-FG 13-mm, polytetrafluoroethylene [PTFE], Millipore Corporation, Billerica, MA, USA). Then, 20 mL of n-heptane was slowly added to the filtered solution as an anti-solvent until precipitation was observed. The precipitates were collected using vacuum filtration and vacuum-dried at 25 °C for 3 h. ITZ amorphous was prepared using a ball milling apparatus MM 301 (Retsch GmbH, Haan, Germany). Briefly, 300 mg of ITZ was milled with two zirconium oxide balls in the 10 mL milling jars for 20 min. The obtained powders were measured using powder X-ray diffractometry (PXRD) and differential scanning calorimetry (DSC).

2.2.2. Preparation of dry powder formulations of ITZ and ITZ cocrystals

ITZ-SA, ITZ-TA, and ITZ powders were jet-milled using a 70AS jet-milling system (Powrex Corporation, Hyogo, Japan). The resultant micronized powders were measured using PXRD and laser diffraction particle size analysis (PSA) using a particle size analyzer, to determine the crystal form and particle size. Micronized D-mannitol was used as a water-soluble wetting agent for the inhalation powder. D-mannitol was dissolved in a mixture of ethanol and water (1:1, v/v) and spray-dried using a nanospray dryer B-90 (Buchi Corporation, New Castle, DE, USA) with spray mesh aperture size of 5.5 μm at 90 °C as the inlet temperature. The spray-dried D-mannitol and each micronized ITZ and ITZ cocrystal powder were physically mixed using a Planet M mixer (Gokin Planetaring Inc., Kanagawa, Japan) to prepare the formulation powders containing 20% (w/w) of the ITZ concentration. The obtained powders were analyzed using PXRD, PSA, SEM, and high-performance liquid chromatography (HPLC).

2.2.3. Preparation of ITZ amorphous inhalation powders

The ITZ amorphous formulation for inhalation was prepared using the nanospray dryer. Briefly, 250 mg of ITZ and 1000 mg of D-mannitol were dissolved in 1000 mL of a mixture of ethanol and water (4:1, v/v). The solution was spray-dried using a spray mesh with an aperture size of 5.5 μm at 90 °C (inlet temperature). The obtained powders were analyzed using PXRD, PSA, SEM, and HPLC.

2.3. Characterization methods

2.3.1. PXRD

PXRD measurements were performed using an Ultima IV Powder X-ray diffractometer (Rigaku Corporation, Tokyo, Japan) at an accelerating voltage of 40 kV and tube current of 50 mA with a Cu Kα source (λ = 0.154 nm) radiation. The powders (~2 mg) or the compressed disc was placed on a silicon sample plate and scanned between 2 and 35° (2θ) at a 6°/min scanning rate.

2.3.2. DSC

DSC was performed using a DSC1 system (Mettler Toledo, Switzerland). The thermograms were obtained at a temperature of 25–200 °C and a heating rate of 5 °C/min under nitrogen gas at a flow rate of 40 mL/min. The powders (~1 mg) were weighed in