European Journal of Pharmaceutics and Biopharmaceutics 87 (2014) 107–113

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research paper

Formulation of itraconazole nanococrystals and evaluation of their bioavailability in dogs

Lieselotte De Smet [a], Lien Saerens [b], Thomas De Beer [b], Robert Carleer [c], Peter Adriaensens [c], Jan Van Bocxlaer [d], Chris Vervaet [a,*], Jean Paul Remon [a]

[a] Laboratory of Pharmaceutical Technology, Ghent University, Ghent, Belgium
[b] Laboratory of Pharmaceutical Process Analytical Technology, Ghent University, Ghent, Belgium
[c] Applied and Analytical Chemistry, Hasselt University, Diepenbeek, Belgium
[d] Laboratory of Medical Biochemistry and Clinical Analysis, Ghent University, Belgium

ARTICLE INFO

Article history:
Received 4 June 2013
Accepted in revised form 23 December 2013
Available online 1 January 2014

Keywords:
Itraconazole
Wet milling
Cocrystals
Nanosuspension
Dicarboxylic acids
Poorly water soluble drugs

ABSTRACT

The aim of the study is to increase the bioavailability of itraconazole (ITRA) using nanosized cocrystals prepared via wet milling of ITRA in combination with dicarboxylic acids. Wet milling was used in order to create a nanosuspension of ITRA in combination with dicarboxylic acids. After spray-drying and bead layering, solid state was characterized by MDSC, XRD, Raman and FT-IR. The release profiles and bioavailability of the nanococrystalline suspension, the spray-dried and bead layered formulation were evaluated. A monodisperse nanosuspension (549 ± 51 nm) of ITRA was developed using adipic acid and Tween®80. Solid state characterization indicated the formation of nanococrystals by hydrogen bounds between the triazole group of ITRA and the carboxyl group of adipic acid. A bioavailability study was performed in dogs. The faster drug release from the nanocrystal-based formulation was reflected in the in vivo results since T_{max} of the formulations was obtained 3 h after administration, while T_{max} of the reference formulation was observed only 6 h after administration. This fast release of ITRA was obtained by a dual concept: manufacturing of nanosized cocrystals of ITRA and adipic acid via wet milling. Formation of stable nanosized cocrystals via this approach seems a good alternative for amorphous systems to increase the solubility and obtain a fast drug release of BCS class II drugs.

1. Introduction

The number of poorly soluble active pharmaceutical ingredients (APIs) has increased in the last 10 years as the majority of innovative drugs in the development pipeline belong to the class II and IV of the biopharmaceutics classification system (BCS) (i.e. they are classified into the two low solubility categories). Since their low solubility and dissolution rate limits the bioavailability after oral administration, formulation development of these compounds is a major challenge [1,2]. Strategies that have been used to increase the solubility of BCS II and IV drugs include the use of inclusion complexes, salts, solid dispersions/solutions, cocrystals, and particle size reduction [3].

Itraconazole (ITRA), a broad-spectrum triazole antifungal agent belonging to BSC class II (aqueous solubility: 1 ng/ml at pH 7 and 5 μg/ml at pH 1) [4], was for more than two decades used in pharmaceutical research as a model drug to illustrate the enhancement of its solubility and bioavailability using different approaches. While the commercially available formulation (Sporanox®) was developed as an amorphous solid dispersion of ITRA in a hydroxypropylmethylcellulose (HPMC) matrix (layered on sugar beads) to enhance its solubility [5], amorphous solid dispersions of ITRA and HPMC were also manufactured by hot melt extrusion [6,7].

Another strategy to increase the solubility of ITRA is by the formation of cocrystals. Pharmaceutical cocrystals are defined as crystalline solids in which at least one of the molecular components is an API combined with another pharmaceutical acceptable molecule, which interact via hydrogen bonding and other weak forces [8,9]. ITRA cocrystals have been identified in combination with dicarboxylic acids (fumaric, succinic, malic and tartaric acid), where the carboxylic acid interacts with the triazole group of ITRA via hydrogen bonds [9]. These cocrystals enhanced the solubility of ITRA to a level comparable to the solubility of the amorphous form. There is a thin line between the definition of a salt and a cocrystal and currently discussions are ongoing in the literature about the definition of a cocrystal. The acid ionization constant, p*K*a, is a commonly employed tool for predicting solid form molecular ionization states. When Δp*K*a (pKa_{base}–pKa_{acid}) is lower than 1, there will be substantially less proton transfer. If this requirement is

* Corresponding author. Ghent University, Laboratory of Pharmaceutical Technology, Harelbekestraat 72, 9000 Ghent, Belgium. Tel.: +32 9 264 80 54; fax: +32 9 222 82 36.
E-mail address: Chris.Vervaet@UGent.be (C. Vervaet).

0939-6411/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejpb.2013.12.016

fulfilled, the API-excipient should be classified as cocrystal. But exceptions on this rule are already noticed as some API-excipient interactions are classified as a cocrystal, although the ΔpKa lays between 1 and 3 [10]. Another definition is that cocrystals are in neutral state and interact via non-ionic interactions [10]. These non-ionic interactions such as hydrogen bounds are very robust, so cocyrstal forms exhibit a high thermal stability and they are also very stable at high relative humidity, where salts are known to be unstable [11]. Although cocrystals can be formed by various methods such as solution crystallization [12,13], slurry conversions [14], ultrasonication [15,16] and melt processes [17,18], (wet or dry) grinding methods [19–21] are often preferred preparation methods based on their simplicity, eco-friendliness and high productivity [22]. Since wet-milling (i.e. mechanical grinding using milling pearls in a liquid medium) is also a typical top-down method to reduce the particle size of poorly water soluble drugs below 1 μm [2], this technique is used in this work to prepare nanosized cocrystals of ITRA, allowing in a single step to nanosize the ITRA particles and simultaneously form ITRA cocrystals to increase its solubility and bioavailability. Therefore, ITRA was milled in combination with dicarboxylic acids (maleic, adipic, glutaric and succinic acid) and with a surfactant (Tween 80®) to form a stable nanosuspenion. As further transformation of the nanosuspension into a solid dosage form is required to enhance its physical stability and to improve patient convenience [23], the ITRA nanosuspensions were transformed in a solid dosage form by spray-drying and bead coating.

2. Materials and methods

2.1. Materials

Adipic, maleic, glutaric and succinic acid were purchased from Sigma (Bornem, Belgium). Polysorbate 80 (Tween® 80) was obtained from Mosselman (Ghlin, Belgium). Itraconazole (ITRA) was kindly donated by Janssen Pharmaceutica (Beerse, Belgium).

2.2. Preparation and characterization of the ITRA nanosuspension

The cocrystalline ITRA nanosuspensions were prepared by a wet milling technique using Tween® 80 as stabilizer. After dissolving the stabilizer in a 20 ml vial containing 5 ml of demineralized water, ITRA (250 mg) was dispersed in this aqueous phase. Different dicarboxylic acids (maleic, adipic, glutaric and succinic acid) were dissolved in different concentrations in the suspension (Table 1). Zirconium oxide beads (amount 30 g, diameter 0.5 mm) were added to the suspension as milling pearls. The vials were placed on a roller-mill (Peira, Beerse, Belgium) and grinding was performed at 150 rpm for 60 h. After milling the nanoparticles were separated from the grinding pearls by sieving.

The mean particle size and polydispersity index (PDI) of the nanosuspensions was determined by photon correlation spectroscopy, using a Zetasizer 3000 (Malvern Instruments, Worcestershire, UK). Prior to analysis, nanosuspensions were diluted with distilled water.

Table 1
Mean particle size (±SD, $n = 3$) and polydispersity index (PDI) of ITRA (5%) nanosuspension after wet milling with different dicarboxylic acids and Tween®80 (1.25%) in 5 ml H_20.

	Content (%)	Size (nm) ± SD	PDI
Succinic acid	8	1045 ± 58	0.32
	16	924 ± 24	0.30
Adipic acid	0	>5000	1
	2	1203 ± 59	0.34
	4	820 ± 12	0.24
	8	549 ± 51	0.20
	16	526 ± 69	0.21
Maleic acid	8	1651 ± 159	0.35
	16	1548 ± 106	0.34
Glutaric acid	8	1538 ± 161	0.21
	16	1492 ± 128	0.21

2.3. Conversion of the nanosuspension into solid dosage forms

2.3.1. Spray-drying

A nanosuspension containing 5% ITRA, 8% adipic acid and 1.25% (w/v) Tween® 80 was selected for further processing via spray-drying. Prior to spray-drying mannitol was added to the nanosuspension in equal amounts compared to the solid fraction, yielding an ITRA concentration of 17.6% (w/w) in the final powder (ITRA-SD2). The nanosuspension, under constant stirring during processing, was spray-dried in a Büchi mini spray-dryer (Model B295, Büchi, Flawil, Switzerland) using a two-fluid nozzle with a 0.7 mm diameter and nitrogen (at 4 bar) as drying medium. The following process conditions were used: liquid feed rate of 3.4 ml/min; inlet air temperature of 120 °C; aspiration level of 80%. The measured outlet temperature was 56 °C. The yield of the spray-drying process was calculated by dividing the obtained powder weight by the theoretical weight of the powder.

2.3.2. Bead-layering

120 ml of a diluted nanosuspension containing 4.2% ITRA, 6.7% adipic acid and 1.0% Tween® 80 (w/v) was layered on 50 g microcrystalline cellulose (MCC) beads (Cellets®500, Pharmtrans Sanac, Switzerland) using an Oystar Hüttlin Mycrolab (Hüttlin, Schopfheim, Germany). The instrument parameters were set as follow: inlet temperature of 66 °C, atomizing air pressure of 0.8 bar, spray rate of 3 g/min. Prior to the bead layering process mannitol was added in equal amounts compared to the solid fraction of the nanosuspension, yielding an ITRA concentration of 17.6% in the layer and 6.4% overall in the beads (ITRA-BL1).

2.4. Characterization

2.4.1. Thermal analysis

The thermal properties of pure compounds, physical mixtures and corresponding spray-dried formulations were analyzed using a differential scanning calorimeter Q2000 (TA instruments, Zellik, Belgium) equipped with a refrigerated cooling system. Samples (±5 mg) were run in Tzero pans (TA instruments, Zellik, Belgium) with an underlying heating rate of 2 °C/min. The modulation period and amplitude were set at 60 s and 0.318 °C, respectively. Dry nitrogen at a flow rate of 50 ml/min was used to purge the DSC cell. The samples were evaluated according to the three cycle analysis (heating, cooling and heating) from 0 to 180 °C. Modulated differential scanning calorimetry (MDSC) data were analyzed using the Universal Analysis 2000 software (TA Instruments, Zellik, Belgium).

2.4.2. X-ray diffraction

The crystallinity of pure compounds, physical mixtures and corresponding spray-dried formulations was determined via X-ray diffraction using a D5000 Cu Kα diffractor ($\lambda = 0.154$ nm) (Siemens, Karlsruhe, Germany) with a voltage of 40 mV in the angular range of $10° < 2\theta < 60°$ using a step scan mode (step width = 0.02°, counting time = 1 s/step).

2.4.3. Raman spectroscopy

A Rxn1 spectrometer (Kaiser Optical Systems, Ann Arbor, MI, USA), equipped with an air-cooled CCD detector (back-illuminated deep depletion design) was used to collect the Raman spectra. The