European Journal of Pharmaceutics and Biopharmaceutics 78 (2011) 377–384

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research paper

Benznidazole microcrystal preparation by solvent change precipitation and *in vivo* evaluation in the treatment of Chagas disease

Flávia Pires Maximiano [a], Lívia Maria de Paula [a], Vivian Paulino Figueiredo [b], Isabel Mayer de Andrade [b], André Talvani [b], Lívia C. Sá-Barreto [c], Maria Terezinha Bahia [b], Marcílio S.S. Cunha-Filho [c,*]

[a] Escola de Farmácia, Universidade Federal de Ouro Preto (UFOP), Ouro Preto-MG, Brazil
[b] Instituto de Ciências Exatas e Biológicas, Universidade Federal de Ouro Preto (UFOP), Ouro Preto-MG, Brazil
[c] Instituto de Ciências da Saúde, Universidade Federal de Mato Grosso (UFMT), Sinop-MT, Brazil

ARTICLE INFO

Article history:
Received 22 November 2010
Accepted in revised form 3 March 2011
Available online 10 March 2011

Keywords:
Benznidazole
Chagas disease
Solvent change precipitation
Dissolution rate
Microcrystals

ABSTRACT

Benznidazole (BNZ) is traditionally used to treat Chagas disease. Despite its common use, BNZ has a poor water solubility and a variable bioavailability. The purpose of this study was to prepare BNZ microcrystals by solvent change precipitation and to study the effects of BNZ micronisation on therapeutic efficiency using a murine model of Chagas disease. The solvent change precipitation procedure was optimised in order to obtain stable and homogeneous particles with a small particle size, high yield and fast dissolution rate. The thermal and crystallographic analysis showed no polymorphic change in the microcrystals, and microscopy confirmed a significant reduction in particle size. A marked improvement in the drug dissolution rate was observed for micronised BNZ particles and BNZ tablets in comparison with untreated BNZ and commercial Rochagan®. *In vivo* studies showed a significant increase in the therapeutic efficacy of the BNZ microparticles, corroborating the dissolution results.

1. Introduction

Benznidazole (BNZ; N-benzyl-2-nitro-1-imidazole-acetamide) is used as the primary therapeutic agent for treating acute Chagas disease [1]. Although this compound can eliminate the symptoms associated with the acute phase and provides a satisfactory cure rate, it is much less effective in the chronic phase of the disease. Unfavourable pharmacokinetic properties, such as poor water solubility, short terminal half-life and limited tissue penetration, lead to irregular oral absorption and promote an erratic bioavailability [2,3].

In order to develop a more effective treatment, some studies have focused on the development of novel methods to administer BNZ, however, without any clear improvement in its therapeutic effect [4,5]. Silva and collaborators developed a ruthenium benzidazole complex more soluble in water and with higher trypanocidal activity than the free molecule. The synthesis of this derivative is still in laboratorial scale and it has an unknown industrial feasibility [6]. A recent study presents the preparation of microparticles of BNZ using chitosan by the coacervation method providing an increase in dissolution rate of drug but without any *in vivo* testing [7].

The limited oral bioavailability of poor water-soluble drugs such as a BNZ is often related to the dissolution rate in the gastro-intestinal tract, which limits drug absorption [8]. Thus, novel methods to enhance drug dissolution are needed. Several techniques exist to improve the drug dissolution profile, such as reducing the particle size, which increases the surface area [9].

Physical methods, such as milling and grinding, are common ways to reduce the particle size [10,11]. However, these mechanical processes have several disadvantages. Oftentimes, particle size uniformity is not achieved due to the large energy input. In addition, disruption of the crystal lattice can cause physical or chemical instability. Furthermore, micronised powders with a higher energetic surface have a poor flow property and a broad size distribution, producing agglomerates with unfavourable solubility properties [9].

To overcome these problems, some recently developed techniques based on naturally grown crystals have been used to reduce particle size [12]. Briefly, drug powder is directly prepared in the micronised state during particle formation without any mechanical size reduction. Microcrystallisation of drugs is archived by a solvent change process that precipitates the drug in the presence of excipients, which cover the particle surface, to inhibit particle growth [13]. This approach is more advantageous than traditional milling techniques because the particle size is more uniform and the powder is less cohesive [14].

* Corresponding author. Instituto de Ciências da Saúde, Campus Universitário de Sinop, Universidade Federal de Mato Grosso (UFMT), Avenida Alexandre Ferronato, 1200, Reserva 35, Setor Industrial, 78557-267 Sinop-MT, Brazil. Tel.: +55 66 35317679; fax: +55 66 35319797.
E-mail address: marciliofarm@hotmail.com (M.S.S. Cunha-Filho).

0939-6411/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2011.03.003

Therefore, the aim of this study was to prepare and characterise BNZ microcrystals produced by the solvent change method and to evaluate the therapeutic efficacy of fast-dissolving tablets produced with micronised BNZ *in vivo*.

2. Materials and methods

2.1. Materials

BNZ powder (lot 13,871; 99% purity) and commercial tablets Rochagan® were obtained from Roche® (Basel, Switzerland).

Hydroxypropilmethylcellulose Methocel® F50 Premium LV (F50), Hydroxypropilmethylcellulose Methocel® K100 Premium LV (K100), Hydroxypropilmethylcellulose Methocel® E10m Premium LV (E10m) and Starch 1500® were furnished by Colorcon® (Cotia, Brazil). Hydroxyethylcellulose Cellosize® QP 300 (HEC) was supplied by Polytechno® (São Paulo, Brazil). Magnesium stearate, polyethylene glycol 4000 Carbovaw® (PEG) and sodium lauryl sulphate (SLS) were obtained from Vetec® (Duque de Caxias, Brazil). All solvents were of analytical grade.

2.2. Solvent change precipitation procedure

2.2.1. Preliminary investigation

BNZ micronisation was performed using the solvent change method by instantaneously mixing two liquids in the presence of a stabilizing agent, as described by Gassmann and co-workers [15]. Briefly, the drug was dissolved close to the saturation concentration in an organic solvent that is miscible with water. A stabilizing agent was dissolved in water. The aqueous solution was poured rapidly from a beaker into the drug solution with constant stirring. The BNZ microsuspension was filtered through a nylon membrane (0.45 μm) and dried in an oven at 37 °C for 24 h.

Preliminary studies were performed to determine the ideal conditions for the preparation of microcrystals using the solvent change method. The effects of experimental variables on microcrystal yield and particle dissolution rate were determined.

Particle stability and the particle's physical properties are dependent on the stabilizer used [9]. Several stabilizers with a hydrophilic structure were investigated (F50, K100, E10m, PEG and HEC), and the BNZ particles produced from them were designated, BNZ_{F50}, BNZ_{K100}, BNZ_{E10m}, BNZ_{PEG} and BNZ_{HEC}, respectively. The organic solvent was chosen based on the ability to dissolve BNZ and on the amount of precipitation obtained after phase change. The solvents tested were acetone, methanol and ethanol. The solution temperature during phase change was also evaluated; the experiment was performed at 5 °C and 25 °C. Four different solvent ratios (organic to water) were tested: 1:0.5, 1:1, 1:4 and 1:8. In each experiment, a high concentration of stabilizer was used (0.1% w/v) in order to not make this a limiting factor.

Finally, four different stabilizer concentrations (0.005, 0.05, 0.01 and 0.1% w/v) were tested in order to estimate the minimum concentration of polymer necessary to obtain the soluble drug particle at a maximum yield. Each experiment was performed in triplicate.

After the preliminary studies, the optimised crystallisation method was used to produce a sufficient amount of micronised particle for the *in vivo* experiment. Approximately 50 g of micronised BNZ was prepared for each stabilizer. Production scale-up was performed fitting the crystallisation condition.

2.3. Particle characterisation

2.3.1. Drug assay

BNZ was quantified using a validated spectrophotometric method. A calibration curve was made using a standard solution of BNZ in the range of 8–28 μg/mL in water/methanol (1:1 v/v) using an UV–visible spectrophotometer (Heλios α Thermo Electron Corporation® Waltham, USA) set to 324 nm. No effect of polymers addition on the UV spectrum of BNZ was verified.

2.3.2. Dissolution studies

Dissolution studies were performed using sink conditions and the USP basket method (apparatus 1) using Nova Ética® 299 dissolution equipment (Vargem Grande Paulista, Brazil). The rate of stirring was 75 ± 2 rpm, and the temperature of the dissolution media was set to 37 ± 1 °C. The dissolution medium (900 mL) was simulated gastric fluid pH 1.2 [16]. BNZ samples of 50 mg or an equivalent amount of each micronised system was placed in hard gelatin capsules and tested in triplicate.

At regular time intervals, suitable amount of sample medium was withdrawn and same amount replaced by fresh medium. Samples were diluted and filtered through a syringe filter (0.45 μm). The concentration of dissolved drug in the medium was determined using a spectrophotometer set to 324 nm. The dissolution profiles were compared using the dissolution efficiency at 20 min [17]. Statistical analysis of the dissolution efficiency was performed using a one-way analysis of variance (ANOVA) followed by least significant difference.

2.3.3. Scanning electron microscopy (SEM)

Scanning electron micrographs were taken using a JEOL JSM-5510® microscope (Westmont, USA) operating at 15 kV. Particles were fixed on a brass stub using a conductive double-sided adhesive tape and coated under vacuum with graphite in an argon atmosphere at 50 mA for 50 s.

2.3.4. Optical microscopy

The particle surface morphology was examined using a TNB-04D OPTON® (São Paulo, Brazil) microscope connected to a video camera.

2.3.5. X-ray powder diffractometry (XRPD)

X-ray powder diffractograms were obtained using a Shimadzu XRD 6000 diffractometer (Kyoto, Japan) equipped with an iron tube and a graphite monochromator. The scans were performed between 2° and 60° (2θ) with a scanning speed of 2°θ/min.

2.3.6. Differential Scanning Calorimetry (DSC)

Samples weighing 2–3 mg were placed in aluminium crucible pans and heated from 25 to 250 °C at a rate of 10 °C/min using a DSC 2010 TA Instrument® (New Castle, USA). Gas was purged using nitrogen at a flux rate of 50 mL/min. The DSC instrument was calibrated using indium and zinc standards.

2.3.7. Surface area

Particle surface area was determined using gas adsorption, and the calculation was based on the BET equation [18]. Samples were degassed under a vacuum for 24 h at 40 °C and then analysed by a Surface Area Analyser Nova 1000 (Quantachrome Instruments®, Boynton Beach, USA).

2.3.8. Flow property study

The flow properties of untreated and micronised samples were evaluated using the Carr index and pharmacopoeia parameters [16,19]. The angle of repose was assessed using the fixed funnel method [20]. Flow time was established by recording the time, and it took for a predetermined sample to flow through a standard funnel.

The compressibility index was determined using a density apparatus and was calculated by the following formula below: