Journal of Drug Delivery Science and Technology 45 (2018) 135–141

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

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

In vivo evaluation of the efficacy, toxicity and biodistribution of PLGA-DMSA nanoparticles loaded with itraconazole for treatment of paracoccidioidomycosis

Elaine P. Cunha-Azevedo[a,c], Karen R. Py-Daniel[a], Marigilson P. Siqueira-Moura[b], Anamélia L. Bocca[a], Maria S.S. Felipe[a], Antonio C. Tedesco[b], Osmindo R. Pires Junior[a], Carolina M. Lucci[a], Ricardo B. Azevedo[a,*]

[a] Biological Sciences Institute, Universidade de Brasília, Brasília DF 70910-900, Brazil
[b] Department of Chemistry, Faculty of Philosophy, Sciences and Letters of Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto SP 14040-901, Brazil
[c] Health and Sciences Biological Institute, Centro Universitário de Belo Horizonte, Belo Horizonte MG 31910-900, Brazil

ARTICLE INFO

Keywords:
Lung mycosis
Polymeric nanoparticles
Dimercaptosuccinic acid
Itraconazole

ABSTRACT

Itraconazole(ITZ) – an antifungal agent of the azole class – is clinically used to treat a variety of fungal infections. Among them, paracoccidioidomycosis (PCM), a fungal infection caused by *Paracoccidioides brasiliensis,* is the most prevalent systemic fungal infection in Brazil as well as in many countries in South America. Conventional treatments of PCM with itraconazole include oral solution and capsule formulations, both associated to many side effects such as nausea, vomiting, abdominal pain, diarrhea, headaches and mild alopecia. Drug delivery systems enables the development of new pharmaceutical formulations for the controlled delivery of drugs, which can avoid many of the problems related to low efficacy and side effects of traditional drugs. In this study, using HPLC, we determined the biodistribution of ITZ administered both, as a nanoparticle presentation (PLGA nanoparticle coated with DMSA – ITZ-NANO), as well as oral solution (Free ITZ) in healthy mice. We also compared the therapeutic efficiency as well as toxicity of both forms of ITZ presentations in the treatment of PCM infected mice using clinical, biochemical and histological approaches. Biodistribution demonstrated higher accumulation of ITZ in lung, liver and spleen of ITZ from ITZ-NANO. ITZ-NANO treatment of chronic PCM was capable of eliminate fungal cells from lungs and avoided the side effects of alopecia and increment of liver enzymes concentration. The proposed treatment, with a lower administration dose of ITZ-NANO and decreased number of administrations, demonstrated promising potential with higher success rate, and less stress for the patient with usage of this nanomaterial.

1. Introduction

Itraconazole (ITZ) – an antifungal agent of the azole class – is clinically used to treat a variety of fungal infections, such as those caused by *Aspergillus spp.*, *Candida spp.*, *Cryptococcus neoformans*, *Blastomyces* spp., *Histoplasma* spp., *Coccidiodes* spp., and *Paracoccidioides* spp. [1], [2] Itraconazole acts by impairing the ergosterol synthesis, essential component of the fungal cell membrane [3].

Paracoccidioidomycosis (PCM), the fungal infection caused by *Paracoccidioides brasiliensis,* is the most prevalent systemic fungal infection in Brazil as well as in many countries in South America, and is reported as the eighth leading cause of mortality from infectious disease among chronic infectious and parasitic diseases [4].

In traditional fungal infection treatments, administration of high doses of itraconazole occurs over long periods, resulting most of time in severe side effects such as nausea, vomiting, abdominal pain, diarrhea, headaches and mild alopecia. It can also cause hepatotoxicity and thereby increase the levels of bilirubin and transaminases [5]. The occurrence of side effects and the toxicity of this drug increase the necessity of different approaches for specific drug delivery and alternative therapeutic protocols [6].

Nanobiotechnology enables the development of new pharmaceutical formulations for controlled delivery of drugs, which can avoid many of the problems related to low efficacy and side effects of traditional drugs. Some of the advantages of using nanoparticles for drug delivery include: enhanced stability, controlled release and enhanced

* Corresponding author. Universidade de Brasília, Biological Sciences Institute, Departamento de Genética e Morfologia, Laboratório de Morfologia e Morfogênese, Campus Darcy Ribeiro, Asa Norte, Brasília DF 70910-900, Brazil.
E-mail address: razevedo@unb.br (R.B. Azevedo).

https://doi.org/10.1016/j.jddst.2018.02.014
Received 2 October 2017; Received in revised form 8 February 2018; Accepted 20 February 2018
Available online 08 March 2018
1773-2247/

bioavailability [7]. Previous studies from our group, with magnetic nanoparticles coated with dimercaptosuccinic acid (DMSA), indicated that this acid is capable of targeting nanoparticles to the lungs [8,9]. A proposed approach is to use such properties of DMSA to target nanoparticles to the lung to treat pulmonary infections such as PCM. Amaral et al. (2009) described efficient treatment of PCM in lungs with amphotericin B loaded nanoparticles coated with DMSA, emphasizing lung-targeting role of DMSA [10].

The aim of this work was to 1) determine the biodistribution of ITZ administered either as a poly(lactic-co-glycolic) acid (PLGA) nanoparticle containing ITZ and coated with DMSA (PLGA nanoparticle coated with DMSA – ITZ-NANO), or ITZ in oral solution presentation (Free ITZ) in healthy mice; 2) to verify the therapeutic efficacy of both forms of ITZ in the treatment of PCM infected mice. Nanoparticle biodistribution was determined by ITZ quantification in different organs by HPLC. To determine therapeutic efficacy, animals were infected with PCM and treated with ITZ-NANO or Free ITZ and clinical, biochemical and histological aspects were analyzed.

2. Material and methods

2.1. Drugs and chemicals

Itraconazole (ITZ), poly(lactic-co-glycolic acid, 50:50) (PLGA 50:50), polyvinyl alcohol (PVA) and dimercaptosuccinic acid (DMSA) were purchased from Sigma Aldrich (Sigma–Aldrich Co., St. Louis, MO, USA) and Itraconazole internal standard (5OR-R51012) was purchased from Fitzgerald (Fitzgerald Industries International Inc., Concord, MA, USA).

Acetonitrile, methanol and tetrahydrofuran used for the HPLC analyses were HPLC grade and purchased from Mallinckrodt (Mallinckrodt Inc., Hazelwood, MO, USA). Trifluoroacetic acid was purchased from VETEC (Duque de Caxias, RJ, Brazil). Milli-Q water was obtained from a *Barnstead* EASYpure** II *Thermo Scientific* (San Jose, CA, USA) and was used to prepare aqueous solutions.

2.2. ITZ-NANO and free ITZ preparation

DMSA-PLGA nanoparticles containing itraconazole (ITZ-NANO) were synthesized and characterized as described by Cunha-Azevedo et al. (2011) [11] and were prepared using a modified emulsification-evaporation technique. Briefly, an organic solution of PLGA and ITZ was prepared. This organic phase was poured slowly into an aqueous solution of polyvinyl alcohol (PVA) 1% (w/v) which was homogenized using an Ultraturrax emulsifier. After this, a double emulsion was formed with 1% PVA (w/v) solution and the organic solvent was removed from the solution by continuous stirring at room temperature inducing polymer precipitation as nanospheres. The nanoparticles were isolated by centrifugation and were washed three times with distilled water. The formulation was suspended in PBS solution. DMSA was adsorbed to PLGA nanoparticle with the final concentration of 0,05 mol/L and stored at 4 °C. All procedures were carried out in a sterile hood. The nanoparticles formed had an average size of 174 nm, efficiency of incorporation of itraconazole into 72,8% and zeta potential of −40 mV [11]. ITZ concentration loaded on PLGA was limited to optimum synthesis conditions of nanoparticles adequate for an intravenous vehicle. Nonetheless, doses used were in accordance with previous publications [12].

An oral solution sugar-free of itraconazole for gavage administration (Free ITZ-"*Xarope sem açúcar*") was manipulated by Farmacotecnica® (Brasilia, DF, Brazil), in order to simulate the conventional treatment. Optimum Free ITZ dosage was chosen based on literature [13].

2.3. Animals

Animals used in this study were female BALB/c mice ageing 10–14 weeks and weighing 20–22 g, purchased from the University of Campinas, SP, Brazil. The mice were housed in polypropylene cages under controlled conditions of luminosity and received food and water *ad libitum*. All animal procedures performed in this study were approved by the Animal Care and Use Committee of the University of Brasília (UnB), Brasília – Federal District, Brazil (UnBDoc: 12155/2007) and European Community guidelines as accepted principles for the use of experimental animals were adhered to.

The study was divided in two major approaches: a biodistribution in healthy animals and the treatment of PCM infected animals.

2.4. ITZ biodistribution

Animals received a single dose of either 1) ITZ-NANO containing 3 μg of ITZ per gram of body weight (administered intraperitoneally) or 2) Free ITZ oral solution with 50 μg of ITZ per gram of body weight (administered by gavage). At 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, and 72 h after administration of ITZ-NANO or Free ITZ (n = 5/group for each treatment/time), animals were anaesthetized (ketamine 60 mg/kg and xylazine 7.5 mg/kg), blood was collected by heart puncture and was transferred to tubes containing EDTA. After 30 min at room temperature, tubes were centrifuged for 5 min at 500 × *g* and plasma was stored at −80 °C for future analyses. After the animal's death, liver, spleen, lung and kidney were excised, washed with cold 0.9% NaCl (w/v) solution, blotted on filter paper, weighted and stored at −80 °C for future analyses.

2.4.1. HPLC instrumentation

The chromatographic equipment (Shimadzu-Prominence, Kyoto, Japan) comprised of on-line degasser (Model DGU $20A_5$), solvent delivery module (Model LC-20AT), autosampler (Model SIL-20AHT), column oven (Model CTO-20A), fluorescence detector (Model RF-10AXL) and system controller CBM-20A. Reverse-phase C_{18} column ACE AQ (25 × 0.4 cm, 5 μm particle size) (ACE, Aberdeen, Scotland) with a pre-column (1 × 0.4 cm, 5 μm particle size). (ACE, Aberdeen, Scotland) were used.

2.4.2. HPLC analysis

The mobile phase was obtained from the mixture of 0.12% (v/v) TFA in Milli-Q water (pump A) and 0.12% (v/v) TFA in acetonitrile (pump B) at 1:1 proportions. Fluorimetric measurements were carried out in a 12 μL flow cell at 260 and 365 nm excitation and emission wavelength, respectively. The injection volume was 10 μL and the flow-rate during the assays was 1 mL/min at working pressure of 80 kgf/cm^2. Analysis was performed using column temperature of 30 °C. Software LCsolution (version 1.24 SP1 Shimadzu, Tokyo, Japan) was used for data processing and identification of chromatographic parameters.

For ITZ quantification in plasma, 200 μL of plasma were transferred to microcentrifuge tubes containing 20 μL of 20% $ZnSO_4$ (w/v), and vortexed for 1 min. Then 700 μL of methanol was added for ITZ extraction, followed by agitation on a vortex for 3 min and centrifugation for 5 min at 7000 × *g*. Liquid phase was transferred to a 2 mL volumetric flask and the process was repeated with 1 mL of methanol. For ITZ quantification in tissue, whole spleen, lung and kidneys and 400 mg of liver (due to large mass of liver) were homogenized individually in 1.5 mL microcentrifuge tubes with hand pestles and extracted with the same process used for plasma. Prior to extraction of plasma and tissue, 40 μL of Itraconazole Internal Standard (0.3 μg/mL) were added to tubes. The use of internal standards reduces errors associated to loss occurred during extraction. Biodistribution of ITZ from ITZ-NANO and Free ITZ was performed using HPLC detection method described and validated by Py-Daniel et al. (2014) [14]. In the cited method, the recovery of ITZ with the methodology described above was established as