Veterinary Parasitology 251 (2018) 78–84

Contents lists available at ScienceDirect

Veterinary Parasitology

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

Research paper

Albendazole nanocrystals in experimental alveolar echinococcosis: Enhanced chemoprophylactic and clinical efficacy in infected mice

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ARTICLE INFO

Keywords:
Alveolar echinococcosis
Echinococcus multilocularis
Albendazole
Nanocrystals

ABSTRACT

Human alveolar echinococcosis is caused by the fox tapeworm *Echinococcus multilocularis* and is usually fatal if left untreated. Medical treatment with albendazole (ABZ) remains an effective option. However, due to its low aqueous solubility, ABZ is poorly and erratically absorbed following oral administration resulting in low drug levels in plasma and liver distribution. Thus, there arises the need to find a simple, efficient and scalable method to produce new ABZ formulations with increased bioavailability. Bearing this in mind, ABZ nanocrystals (ABZ-NCs) appears to be a useful tool to achieve this goal. The aim of the current study was to investigate the chemoprophylactic and clinical efficacy of an ABZ-NC formulation on mice infected with *E. multilocularis*. In the chemoprophylactic efficacy study, mean weight of the cysts recovered from the ABZ-NC group was 50% lower than that recorded from untreated mice, whereas the treatment with ABZ suspension did not show preventive effect. The viability of protoscoleces isolated from ABZ-NC treated mice was significantly lower than control groups. In the clinical efficacy studies, both ABZ formulations resulted in a reduction in the mean weight of the cysts obtained from mice, however only the treatment with the nanosuspension revealed significant differences ($P < 0.05$) compared to the control groups. Treatment with ABZ-NCs reduced the weight of the cysts by 77% and the viability of their protoscoleces to 34%. All these results coincided with the tissue damage determined at the ultrastructural level. The enhanced chemoprophylactic and clinical efficacy of ABZ-NCs observed in this study could be attributed to an increase in the oral bioavailability of the drug. In a next step, we will characterize the cyst concentration profile after the administration of ABZ-NCs in mice infected with *E. multilocularis*.

1. Introduction

Alveolar echinococcosis (AE) is a serious helminthic zoonosis caused by the metacestode stage of *Echinococcus multilocularis*. This parasitic infection can lead to severe damage to the human liver, lungs and other organs, and is potentially lethal disease when left untreated. Infection of intermediate host such as rodents or accidentally humans is initiated by oral uptake of infectious eggs, which contain the oncosphere larva. After hatching in the host intestine, oncospheres penetrate the intestinal wall and use the blood and lymphatic system for dissemination. They typically invade the liver, where they develop into the metacestode stage. *E. multilocularis* metacestodes proliferate asexually by forming small daughter vesicles, leading to a parasite mass that exhibits tumor-like properties and progressively infiltrates the neighboring tissue (Kern, 2010).

The major treatment option for AE is radical surgery accompanied by pre- and post-operative medical treatment with albendazole (ABZ). Operative resection of lesions is frequently incomplete because of the tumor-like proliferation and the multilocular aspect of the parasite. In cases where surgery is not possible, medical treatment with benzimidazole methyl carbamates such as mebendazole and ABZ remains an effective option. ABZ is the most common and effective antiparasitic

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https://doi.org/10.1016/j.vetpar.2017.12.022
Received 9 January 2017; Received in revised form 27 December 2017; Accepted 29 December 2017

drug for AE treatment (Brunetti et al., 2010). However, due to its low aqueous solubility (0.2 μg/ml in water at 25 °C), ABZ is poorly and erratically absorbed following oral administration resulting in low drug levels in plasma and liver distribution (Jung et al., 1998; Daniel-Mwambete et al., 2004). Consequently, this compound has to be administrated at high or multiple doses in order to provide therapeutic concentrations, causing adverse effects in some cases (Brunetti et al., 2010). On the other hand, the poor water solubility of ABZ offers only few formulation possibilities, limiting the administration routes (Alanazi et al., 2007).

Whereas permeability is an intrinsic drug property that is hard to modify, different techniques have been developed which can improve ABZ water solubility and dissolution rate, such as the formulation of solid dispersions (Castro et al., 2012), oil/water emulsion (Mingjie et al., 2002, Shuhua et al., 2002), incorporation into liposomes (Wen et al., 1996), cyclodextrin complexes (Palomares-Alonso et al., 2010), co-grinding (Vogt et al., 2008) and chitosan-microspheres (Abulaihaiti et al., 2015). Several clinical studies have demonstrated that enhanced systemic availability of the parent drug/active metabolite obtained by increased drug absorption correlates with an improved antiparasitic effect (Wen et al., 1996; Mingjie et al., 2002; Shuhua et al., 2002; Dvoroznáková et al., 2004; Ceballos et al., 2008; Palomares-Alonso et al., 2010; Pensel et al., 2014, 2015; Abulaihaiti et al., 2015).

Most of the strategies described above have shown limited success in the improvement of ABZ bioavailability (Paredes et al., 2016). Besides, these formulation approaches have relatively low drug loading efficiency and manufacturing process are complicated (Sun and Yeo, 2012). In this context, the formulation of drug nanocrystals (NCs) has emerged as a very promising tool for the formulation of poorly soluble drugs.

By definition, drug NCs are nanoparticles being composed of 100% drug, being generally stabilized by surfactants or polymeric steric agents and according to the definition of nanoparticles the mean particle size is below 1 μm. NCs are generally produced in an aqueous medium as nanosuspensions and then, further solvent removal might be necessary in order to obtain redispersible powders (Müller et al., 2011).

According to the Noyes–Whitney and Ostwald–Freundlich equations, a decrease in particle size lead to an increase in the specific surface, enhancing the drug dissolution rate and the saturation solubility (Gao et al., 2012). Moreover, the increase in the contact area leads to an increase in the mucoadhesiveness of nanomaterial. Therefore drug NCs display series of benefits in oral application of poorly soluble drugs, including improved oral absorption, higher bioavailability, rapid action onset, reduced fed/fasted state variability and reduced intersubject variability (Shegokar and Müller, 2010).

Recently, Paredes et al. (2016) produced ABZ-NCs with a final particle size of approximately 500 nm by an optimized methodology. ABZ formulated as powdered self-dispersible NCs presented a high redispersion capacity, as well as enhanced saturation concentration and dissolution rate. For orally administered drugs, optimization of these two properties is relevant (Paredes et al., 2016). Oral administration of ABZ-NCs increased the drug plasma levels in dogs and healthy mice (Paredes et al., 2017; 2018). Moreover, this improved pharmacokinetic performance observed for ABZ-NC formulation correlated with an improved in vivo therapeutic response against a model intestinal haematophagous nematode parasite in dogs (Paredes et al., 2018). The aim of the current study was to investigate the chemoprophylactic and clinical efficacy of an ABZ-NC formulation on mice infected with *E. multilocularis* metacestodes.

2. Materials and methods

2.1. Chemicals

ABZ was purchased from Todo Droga, Argentina. The stabilizer Poloxamer 188 (P188) was obtained from Rumapel, Argentina. All assays were performed using Milli-Q® water (Merck Millipore, USA).

2.2. Nanocrystal formulation

The ABZ nanosuspension was prepared by high pressure homogenization and further water removal was carried out by spray drying as described by (Paredes et al., 2016). Briefly, 5 g of ABZ and 5 g of P188 were ground in a mortar; water (190 ml) was gradually added until a homogeneous suspension was obtained. Afterwards, the sample was processed in a high pressure homogenizer by 30 cycles at 1200 bar (Avestin C5 Emulsiflex®, Canada). Samples were cooled using a heat exchanger with counter-flow cold water (5 °C) during the homogenization process. The obtained nanosuspension was spray-dried (Mini Spray Dryer B-290, Büchi Labortechnik AG, Switzerland) using a two-fluid nozzle with a cap orifice diameter of 1.5 mm with the operating conditions being: atomization air (L/h): 819, aspiration (m^3/h): 30, temperature (°C): 45 and feed pump (ml/min): 2. The obtained powder of ABZ: P188 (1:1) was used for the efficacy studies.

2.3. ABZ formulations

ABZ suspension (0.5 mg/ml) was prepared by dispersion of ABZ pure standard in MilliQ water (pH = 7.0) under shaking (12 h). The nanosupension (0.75 mg/ml) was prepared by dissolution of ABZ-NCs in Milli-Q® water (pH = 7.0) under shaking. The formulations were vigorously shaken before its intragastric administration to mice.

2.4. Ethic statement

Animal procedures and management protocols were approved by the Institutional Animal Care and Use Committee (RD 148/15) of the Faculty of Exact and Natural Sciences, National University of Mar del Plata, Argentina and carried out in accordance with the revised form of The Guide for the Care and Use of Laboratory Animals (National Research Council US, 2011). Unnecessary animal suffering was avoided throughout the study. Female CF-1 mice (body weight 25 ± 5 g) were used. The animals were housed in a temperature-controlled (22 ± 1 °C), light-cycled (12-h light/dark cycle) room. Food and water were given ad libitum.

2.5. Parasite material

All experiments were carried out using parasite isolate 8065 (kindly provided by Klaus Brehm, Institute for Hygiene and Microbiology, University of Würzburg). Cystic mass dissected from experimentally infected female CF-1 mice was pressed through a metal tea strainer and the suspension obtained was washed several times with an antibiotic solution and maintained in the same solution overnight before intraperitoneal inoculation into mice (Albani et al., 2015).

2.6. Experimental design

Eighty mice were infected by intraperitoneal inoculation with 0.5 ml of homogenized metacestode material. Two different experimental designs were conducted: a chemoprophylactic efficacy study and a clinical efficacy study.

2.6.1. Chemoprophylactic efficacy study

Twenty four hours after the infection, mice (n = 40) were allocated into 4 experimental groups (10 animals/group) and treated as follows: a) Control group, animals receiving Milli-Q® water as a placebo; b) Blank-NC group, animals receiving excipients in Milli-Q® water; c) ABZ suspension treated group; d) ABZ-NC treated group. Treatments were performed daily for 30 days by intragastric administration at the ABZ dose of 5 mg/kg. Eleven weeks after infection, mice were euthanized, and necropsy was carried out immediately thereafter.