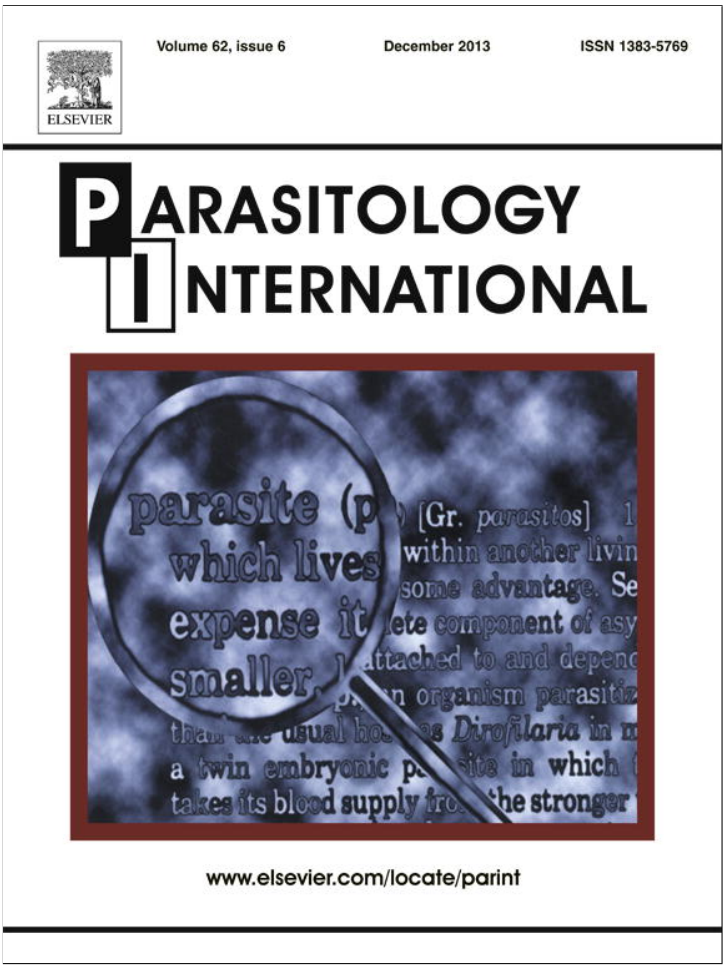

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Short communication

Novel albendazole formulations given during the intestinal phase of *Trichinella spiralis* infection reduce effectively parasitic muscle burden in mice

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ABSTRACT

Trichinellosis is a zoonotic disease affecting people all over the world, for which there is no speedy and reliable treatment. Albendazole (ABZ), an inexpensive benzimidazole used in oral chemotherapy against helminthic diseases, has a broad spectrum activity and is well tolerated. However, the low absorption and variable bioavailability of the drug due to its low aqueous solubility are serious disadvantages for a successful therapy. In this study, we evaluated the *in vivo* antiparasitic activity of three novel solid microencapsulated formulations, designed to improve ABZ dissolution rate, in a murine model of trichinellosis. Both ABZ and the microparticulate formulations were administered during the intestinal phase of the parasite cycle, on days 5 and 6 post-infection. This protocol significantly decreased muscle larval burden measured in the parenteral phase, on day 30 post-infection, when compared with the untreated control. Moreover, two of the three microencapsulated formulations both strongly and consistently reduced worm burden.

1. Introduction

Trichinellosis, a parasitic disease caused by the nematode *Trichinella* spp., is one of the main food-borne zoonoses that remain being a threat worldwide, despite concerted control efforts [1]. As reported by Pozio and Murrel [2], infection by *Trichinella* spp. has been detected in domestic and/or wild animals from all the continents, with the exception of Antarctica, where there is no record of the parasite; and the species most usually reported in humans is *Trichinella spiralis*. This nematode has a complex life cycle in which the same host harbors different forms of the parasite.

Human trichinellosis has been documented in over 50 countries and is classically characterized by gastroenteritis, myalgia, malaise, facial edema, headache, subungual or conjunctival hemorrhages, and increased eosinophils, leukocytes, and muscle enzymes [3]. Whether trichinellosis is a low-prevalence disease or is frequently misdiagnosed is currently unclear. Its detection can be difficult in low-level infections and its clinical manifestations overlap those of many other common diseases, such as influenza or rheumatic diseases [4]. In humans and animals, generally, the infection is the result of the consumption of raw or poorly cooked meat or meat products from infected animals. Once the meat is in the digestive tract, the enzymes release the infective first stage larvae (L1 larvae). These larvae mature and mate within 24–48 h post-infection in the intestine of host. The release of newborn larvae begins on day 5 and they migrate through blood and lymphatic vessels, reach striated muscle fibers, actively penetrate inside, grow, mature, generate a remarkable phenomenon of adaptation to its host cell and finally encapsulate in the muscle around day 30 post-infection [5]. The selected therapeutic agent to treat this infection is albendazole (ABZ). ABZ is a benzimidazole derivative with a broad spectrum activity against intestinal and systemic parasitoses [6–9]. However, ABZ is classified as class II by the Biopharmaceutics Classification System (BCS) because it has an extremely poor aqueous solubility (1 μg/mL) that limits oral absorption [10–12]. Thus, its bioavailability is very low and erratic. Furthermore, this aqueous insolubility reduces the pharmaceutical alternatives for drug formulation and administration, especially for pediatric patients. Alternatives for improving the dissolution rate and solubility of poorly

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soluble compounds are to increase the surface area by decreasing particle size and to optimize the wetting characteristics of the surface. [13]. Different strategies have been studied to modify ABZ solubility and bioavailability properties, such as the preparation of solid dispersions, the use of surfactants [14], the complexation with cyclodextrins [15] and the formulation of microspheres and microparticles [16]. One of the microencapsulation polymers proposed for this study is chitosan (CH), a biodegradable cationic polysaccharide obtained by chitin deacetylation. The microspheres used in this assay were prepared by a modified spray drying methodology, based on the ionic gelation process with the anionic surfactant sodium lauryl sulfate (SLS) and polymers like pectin (P) and sodium carboxymethyl cellulose (CMC) [17,18].

The aim of this study was to compare the *in vivo* intestinal antihelminthic effectiveness of formulations obtained by different microparticle manufacturing procedures, designed to enhance albendazole solubility, in a murine model of trichinellosis.

2. Preparation of the microparticulate formulations

ABZ, CMC, P, and SLS were supplied by Sigma-Aldrich Chemie GmbH (Steinheim, Germany), and CH was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA).

MP1. 100 mg ABZ was dissolved at room temperature in 30 mL of glacial acetic acid, and distilled water was added to obtain a concentration of 30% v/v. CH (0.5% w/v) was dispersed in the ABZ–acetic acid solution. The SLS solution (0.2% w/v; pH 8) was prepared by dissolving in 100 mL of water under stirring for a fixed time (2 h). The polymer solutions were heated at 45 °C and the binary system SLS was then dropped slowly over the ABZ–CH solution to avoid aggregate formation.

MP2. ABZ (100 mg) was dissolved at room temperature in acetic acid (25 mL) and water (25 mL); CH (3.0% w/v) was dispersed in the acidic solution. The resulting suspension was stirred to allow complete CH dissolution in the acidic medium. The microspheres were formed by spraying the ABZ–CH solution through a nozzle (0.30 mm in diameter) into a precipitation bath containing the SLS solution (5.00% w/v, pH 8), under magnetic stirring. The polymeric microparticles were filtrated, washed with water, centrifuged twice and finally collected in a drying chamber at 40 °C.

MP3. ABZ (100 mg) was dissolved at room temperature in 30 mL of glacial acetic acid, and distilled water was added to obtain a concentration of 30% v/v. CH (1.0% w/v) was dispersed in the ABZ–acetic acid solution. The CMC (0.2% w/v) and P (0.1% w/v) solutions were prepared by dissolving in 100 mL of water under stirring for a fixed time (2 h). The polymer solutions were heated at 45 °C and the binary system CMC-P was then dropped slowly over the ABZ-CH solution to avoid aggregate formation. The spraying procedure was carried out with a Buchi Mini dryer B-290. The following parameters remained constant: air flow rate 38 m^3/h, feed rate 5 mL/min and aspirator set at 100%. The spray-drying inlet temperature was set at 130 °C; the recorded outlet temperature was 70 °C.

The yield, encapsulation efficiency, and dissolution profiles were performed as described by Leonardi et al. [16].

3. Antiparasitic activity assay in *T. spiralis* infected mice

Susceptible CBi+ mice [19,20], of the CBi colony from the Animal Facilities of the Instituto de Genética Experimental, Facultad de Ciencias Médicas, Universidad Nacional de Rosario (from here on, CBi-IGE stock), were used to evaluate the *in vivo* effect of ABZ formulations given during the intestinal phase of the infection. The CBi-IGE stock comprises five genetically distinct lines; four of the lines (CBi+, CBi−, CBi/C and CBi/L) resulted from selection experiments for body conformation while the fifth (CBi) was the unselected control. A description of the selective procedure is given elsewhere [20]. The lines are currently in their 120th generation of selective breeding. The facilities belong to the conventional, semi-open type. Young adults are housed, separated by sex since weaning, in polypropylene cages (32 cm × 24 cm × 10 cm) with wood shavings for bedding, in a room with constant temperature (24 ± 2 °C), with a relative humidity of 50 ± 10% and a light–dark illumination cycle of 12 h. Cages and bedding are changed twice a week. Tap water and food (Cargill Laboratory Chow, pelletized) are provided *ad libitum*.

Adult CBi+ males (90–100 days old) were weighed (mean weight 54 ± 3 g) and orally infected with 2 *T. spiralis* infective L1 larvae per g of body weight. The larvae used in the infection were recovered by artificial digestion at 37 °C in a 1% pepsin–1% HCl solution, from the muscles of a CBi mouse infected 2–3 months earlier for that purpose. The infection dose for each animal was prepared by counting individual larvae. After infection, the animals were divided into five groups (n = 4 per group): I) ABZ, II) MP1, III) MP2, IV) MP3, and V) infection control without treatment, to test the effect of the different ABZ formulations on *T. spiralis* infectivity. Groups I, II, III and IV were treated with 50 mg ABZ per kg of body weight on days 5 and 6 post-infection. The antihelminthic activity of each ABZ preparation was assessed on the chronic phase of the infection (32 ± 2 days post-infection), by counting the number of muscle encysted larvae (muscle larval load) in the tongue of each animal. Briefly, after euthanizing the mouse, the tongue was excised, weighed and submitted to digestion in 2 mL of digestion fluid (pepsin 1:1000, 0.7 g; HCl, 0.9 g; water, up to 100 mL). The mixture was incubated at 37 °C overnight and stirred periodically at moderate speed. At the end of the incubation period digestion was stopped by adding saline to complete 14 mL and the larvae were allowed to decant for 1 h. The supernatant was removed immediately, and 5 mL of 10% v/v formalin was admixed in order to inactivate the larvae and preserve their structure. Finally, all larvae on each sample were counted under a microscope with a 40× magnification. Larval load was calculated as the total number of larvae per g of muscle tissue (LLr). The reduction percentage of LLr in the treated animals was calculated as follows:

$$\text{Mouse "A" LLr percentage reduction} = \frac{\text{LLr control group mean} - \text{mouse "A" LLr} \times 100}{\text{LLr control group mean}}.$$

All the experiments with mice were done during the first half of the light cycle and according to the animal care standards of the institution, which complies with the guidelines established by the Institute for Laboratory Animal Resources [21]. The protocol was approved by the Animal Care and Use Committee of the National University of Rosario (Permit Number: 648/2012).

Physicochemical studies of the microparticles showed that the dissolution behavior characterized by a slow release at the initial stage

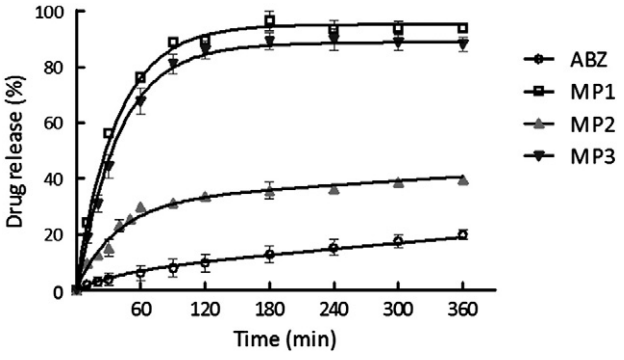

Fig. 1. Dissolution profile of ABZ in HCl 0.1 N at 37 °C of 100 mg ABZ pure drug and the same dosage prepared according to MP1, MP2 and MP3. Each point represents the mean of three measurements ± one standard deviation.

Table 1
Efficacy of different ABZ formulations on muscle larval load[c] of *Trichinella spiralis* in CBi + males.

Group	Relative larval load[a]	Larval load reduction percentage[b]
I (ABZ)	658 ± 139[#]	75 (67–90)[#]
II (MP1)	405 ± 60[#]	86 (81–90)[#]
III (MP2)	592 ± 154[#]	78 (69–92)[#]
IV (MP3)	405 ± 28[#]	86 (83–88)[#]
V(IC)	2853 ± 620[♣]	----

Differences among groups were evaluated by an analysis of variance, using the Bonferroni post-test for comparisons between groups (Relative larval load), or by the non parametric Kruskall–Wallis test, and Dunn's test for between groups comparison (Larval load reduction percentage).
For each variable, differences between groups not sharing the same superscript are significant at the 0.001 level.
[a] Mean ± SEM.
[b] Median (range).
[c] Larval load was measured in the tongue, the preferred site of encystment in mice.

was followed by an increased release rate at a later stage. ABZ's poor solubility in water (1 μg/mL) is reflected in the extent of drug dissolved after 60 min (7%). At variance, the dissolution rate of all the ABZ formulations was higher than that of the unencapsulated drug, ranging from 29 to 77% (Fig. 1).

The dissolution rate of a drug is determined by two critical parameters, size and sphericity, which improve its wettability. The size and shape of the microparticles were evaluated by scanning electron microscopy; MP1 and MP3 were smaller (5 μm) and showed spherical shapes and smooth surfaces, as opposed to MP2. MP2 particles were large, with an average measurement of 50 μm in diameter, and showed irregular shapes.

Table 1 shows the effect of the different ABZ formulations on the parasitic burden of the treated animals. ABZ treatment, in any of its formulations, was associated with a significant decrease in the number of muscle encysted *T. spiralis* larvae, as compared with the infection control (group IV). This result was confirmed by the high percentage reduction seen in the relative larval load in all treated groups. No significant differences in worm load or percentage reduction were observed among the treated groups. Nevertheless, it should be noted that MP1 and MP3 treated mice had the lowest parasitic burden as well as the lowest variance, which denoted homogeneous treatment results. This uniform therapeutic response might be due to an improvement on the intestinal bioavailability of the drug in these formulations as derived from the dissolution test (Fig. 1).

4. Conclusions

In the present study MP1, MP2, and MP3 microparticles containing ABZ were prepared by different spray drying methods, using CH and counter ions. These compounds comply with a basic requirement for an excipient in order to be used in humans or animals, no toxicity for biological environments. In a previous experiment [22], we demonstrated that the formulations without ABZ (placebo) had no detrimental effects on mice. Oral administration of the microparticles neither produced detectable macroscopic effects nor changed survival rate in mice when compared with non-treated animals.

The microparticulate drug delivery systems designed improved the dissolution rate of ABZ, a class II compound with a very poor solubility. MP1 and MP3 had smooth surfaces and smaller size than MP2. *In vitro* dissolution profiles indicated that MP1 and MP3 had a faster release rate than MP2 and the unencapsulated drug. The results described in this study suggest that ABZ–microparticle formulations, particularly MP1 and MP3, showed a significant increase in the dissolution rate, and, therefore, they greatly improved their intestinal antihelminthic efficacy compared with that of ABZ alone (11% increase in the reduction percentage of the parasitic burden). Moreover, mice given MP1 and MP3 showed the lowest mean parasitic burden as well as the lowest variance. This homogenous response observed in MP1 and MP3 treated mice may be associated to a better bioavailability of ABZ. Thus, these microparticle formulations may provide a useful therapeutic alternative for poorly water soluble compounds, improving the drug dissolution rate and, consequently, its absorption by intestinal *T. spiralis* worms.

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References

[1] Pozio E. World distribution of *Trichinella* spp. infections in animals and humans. Veterinary Parasitology 2007;149:3–21.
[2] Pozio E, Murrell KD. Systematics and epidemiology of *Trichinella*. Advances in Parasitology 2006;63:367–9.
[3] Murrell KD, Pozio E. Worldwide occurrence and impact of human trichinellosis, 1986–2009. Emerging Infectious Diseases 2011;17:2194–202.
[4] Kociecka W. Trichinellosis: human disease, diagnosis and treatment. Veterinary Parasitology 2000;93:365–83.
[5] Despommier DD, Gwadz RW, Hotez PJ, Knirsch CA. Parasitic Diseases. 5th ed. New York: Apple Tree Productions; 2005.
[6] Horton J. Albendazole: a broad spectrum anthelminthic for treatment of individuals and populations. Current Opinion in Infectious Diseases 2002;15:599–608.
[7] Casulli A, Gomez Morales MA, Gallinella B, Turchetto L, Pozio E. 2-Hydroxypropyl-β-cyclodextrin improves the effectiveness of albendazole against encapsulated larvae of *Trichinella spiralis* in a murine model. Journal of Antimicrobial Chemotherapy 2006;58:886–90.
[8] Lopez Garcia ML, Torrado S, Torrado J, Martinez Fernadez A, Bolas Fernandez F. Albendazole versus ricobendazole against enteral and parenteral stages of *Trichinella spiralis* in mice. International Journal for Parasitology 1997;27:781–5.
[9] Dayan AD. Albendazole, mebendazole and praziquantel. Review of non-clinical toxicity and pharmacokinetics. Acta Tropica 2003;86:141–59.
[10] Lindenberg M, Kopp S, Dressman JB. Classification of orally administered drugs on the World Health Organization Model list of Essential Medicines according to the biopharmaceutics classification system. European Journal of Pharmaceutics and Biopharmaceutics 2004;58:265–78.
[11] Vogt M, Kunath K, Dressman JB. Dissolution improvement of four poorly water soluble drugs by cogrinding with commonly used excipients. European Journal of Pharmaceutics and Biopharmaceutics 2008;68:330–7.
[12] Mwambete KD, Torrado S, Cuesta-Bandera C, Ponce-Gordo F, Torrado JJ. The effect of solubilization on the oral bioavailability of three benzimidazole carbamate drugs. International Journal of Pharmacy and Pharmaceutical Sciences 2004;272:29–36.
[13] Alanazi FK, El-Badry M, Ahmed MO, Alsarra IA. Improvement of albendazole dissolution by preparing microparticles using spray-drying technique. Scientia Pharmaceutica 2007;75:63–79.
[14] Dib A, Palma S, Suárez G, Farías C, Cabrera P, Castro S, et al. Albendazole sulphoxide kinetic disposition after treatment with different formulations in dogs. Journal of Veterinary Pharmacology and Therapeutics 2011;34:136–41.
[15] Palomares Alonso F, Gonzalez C, Berbard-Bernard MJ, Castillo Montiel MD, Palencia Hernandez G. Two novel ternary albendazole–cyclodextrin–polymer system: dissolution, bioavailability and efficacy against *Taenia crassiceps* cyst. Acta Tropica 2010;113:56–60.
[16] Leonardi D, Lamas MC, Olivieri AC. Multiresponse optimization of the properties of albendazole–chitosan microparticles. Journal of Pharmaceutical and Biomedical Analysis 2008;48:802–7.
[17] Taha MO, Nasser W, Ardakani A, AlKhatib HS. Sodium lauryl sulfate impedes drug release from zinc-crosslinked alginate beads: switching from enteric coating release into biphasic profiles. International Journal of Pharmaceutics 2008;350:291–300.
[18] Fouad EA, El-Badry M, Mahrous GM, Alanazi FK, Neau SH, Alsarra IA. The use of spray-drying to enhance celecoxib solubility. Drug Development and Industrial Pharmacy 2011;37:1463–72.
[19] Vasconi MD, Malfante P, Bassi A, Giudici C, Revelli S, Di Masso R, et al. Phenotypic differences on the outcome of the host–parasite relationship: behavior of mice of the CBi stock in natural and experimental infections. Veterinary Parasitology 2008;153:157–63.
[20] Hinrichsen LI, Di Masso RJ. Use of a murine model original of Argentina to characterize complex phenotypes. Journal of Basic and Applied Genetics 2010;21(7):1–12.
[21] Guide for the care and use of laboratory animals. National Research Council8th ed. Washington, D.C.: The National Academies Press; 2011
[22] Barrera MG, Leonardi D, Bolmaro RE, Echenique CG, Olivieri AC, Salomon CJ, et al. *In vivo* evaluation of albendazole microspheres for the treatment of *Toxocara canis* larva migrans. European Journal of Pharmaceutics and Biopharmaceutics 2010;75(3):451–4.