Inclusion complex and nanoclusters of cyclodextrin to increase the solubility and efficacy of albendazole

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Abstract
Albendazole (ABZ), a benzimidazole widely used to control gastrointestinal parasites, is poorly soluble in water, resulting in variable and incomplete bioavailability. This has favored the appearance ABZ-resistant nematodes and, consequently, an increase in its clinical ineffectiveness. Among the pharmaceutical techniques developed to increase drug efficacy, cyclodextrins (CDs) and other polymers have been extensively used with water-insoluble pharmaceutical drugs to increase their solubility and availability. Our objective was to prepare ABZ formulations, including β-cyclodextrin (βCD) or hydroxypropyl-β-cyclodextrin (HPβCD), associated or not to the water-soluble polymer polyvinylpyrrolidone (PVP). These formulations had their solubility and anthelmintic effect both evaluated in vitro. Also, their anthelmintic efficacy was evaluated in lambs naturally infected with gastrointestinal nematodes (GIN) through the fecal egg count (FEC) reduction test. In vitro, the complex ABZ/HPβCD had higher solubility than ABZ/βCD. The addition of PVP to the complexes increased solubility and dissolution rates more effectively for ABZ/HPβCD than for ABZ/βCD. In vivo, 48 lambs naturally infected with GIN were divided into six experimental groups: control, ABZ, ABZ/βCD, ABZ/βCD-PVP, ABZ/HPβCD, and ABZ/HPβCD-PVP. Each treated animal received 10 mg/kg of body weight (based on the ABZ dose) for three consecutive days. After 10 days of the last administered dose, treatment efficacy was calculated. The efficacy values were as follows: ABZ (70.33%), ABZ/βCD (85.33%), ABZ/βCD-PVP (82.86%), ABZ/HPβCD (78.37%), and ABZ/HPβCD-PVP (43.79%). In vitro, ABZ/HPβCD and ABZ/HPβCD-PVP had high solubility and dissolution rates. In vivo, although the efficacies of ABZ/βCD, ABZ/βCD-PVP, and ABZ/HPβCD increased slightly when compared to pure ABZ, this increase was not significant (P>0.05).

Keywords Albendazole · Inclusion complex · Cyclodextrin · Solubility · Anthelmintic · Sheep

Introduction
Anthelmintic resistance has been recognized as the key limitation for continued productivity and sustainability of livestock production (Woodgate et al. 2017). These authors cite several others who reported that resistance to multiple anthelmintics is now common in sheep gastrointestinal nematodes worldwide. Anthelmintic drugs continue to be the prevalent method to control gastrointestinal parasite infections in sheep (Chagas and Vieira 2007) but are quickly losing their efficacy due to the emergence of parasite resistance (Woodgate et al. 2017).

The existence of anthelmintic-resistant nematodes has been described for decades and has become a problem in small ruminant production, where resistance to most broad-spectrum anthelmintics has been established (Waller 1997). Once the anthelmintic resistance is established in the herd, it is necessary to find new affordable and effective strategies that can be used over a long period. Strategies involving drug delivery systems are a promising way to improve the therapeutic effect of existing veterinary drugs (Prietsch et al. 2014).
Albendazole (ABZ) belongs to the benzimidazole class and is widely used to control gastrointestinal parasites in cattle and sheep for several decades (Lanusse et al. 2014). In susceptible parasites, ABZ binds to free parasite β-tubulin, inhibiting polymerization of microtubules and leading to changes in the energetic metabolism of parasites causing paralysis and starvation (Martin 1997). Like most benzimidazoles, ABZ is poorly soluble in water, presenting variable and incomplete bioavailability, which has favored the appearance of resistance and consequently clinical ineffectiveness (Gokbulut et al. 2007). Bioavailability is one of the determinants of drug efficacy (Dezani 2010). Small differences in solubility may cause significant effects on the absorption and consequently the bioavailability of these substances (Daniel-Mwambete et al. 2004).

Several techniques have been employed to compensate for the low solubility and low bioavailability of drugs, including the use of amorphous solids, nanoparticles, microemulsions, solid dispersions, formation of salts or co-crystals, and the formation of water-soluble complexes (Evrard et al. 2002; Kawabata et al. 2011).

Cyclodextrins (CDs) have played an important role in the development of poorly soluble drugs by increasing apparent solubility and/or dissolution due to their property of forming inclusion complexes or as hydrophilic drug carriers (Challa et al. 2005; Kawabata et al. 2011). CDs are cyclic oligosaccharides (also called cycloamyloses) containing six (α), seven (β), or eight (γ) 1,4-glucopyranose units. They contain a hydrophilic outer surface and a hydrophobic central cavity, which can accommodate a variety of lipophilic drugs. Besides the formation of inclusion complexes, CDs have the ability to self-organize and form nanometric aggregates that may contribute to the solubilization of poorly soluble drugs. These characteristics make CDs an important excipient that modulates the release of complexed drugs, considering that the permanence of the active ingredient at the site of action is an important factor for clinical efficacy (Messner et al. 2010; Kurkov and Loftsson 2013).

Although natural CDs are widely used in research and development of pharmaceutical formulations, they have limitations. CD properties (carrier and solubilizer) can be improved by increasing their number of hydroxyl groups, thus increasing hydrophilicity. The hydroxyalkylated derivatives (such as 2-hydroxypropyl, HPβCD and HPy/CD) are highly water soluble and less hygroscopic than the respective natural CD (Kurkov and Loftsson 2013).

The formation of inclusion complexes occurs by the replacement of water molecules of CD in the host molecule (ABZ), which should have compatible polarity, size, and shape. The efficiency of complexing of CDs is relatively low, and therefore high amounts of CDs are required to achieve the desired complexation, solubilization, and stabilization effect ( Carrier et al. 2007). The complexation efficiency of CDs can be increased by adding the water-soluble polymer (polyvinylpyrrolidone, PVP), resulting in the formation of a multicomponent complex [(drug-CD) n-polymer] (Soares-Sobrinho et al. 2012). PVP affects this association by increasing solubility and stability of the active ingredient, which leads to a smaller concentration of CD in formulation (Loftsson 2007).

The objective of this study was to evaluate the solubility and dissolution of Albendazole and inclusion complexes with two cyclodextrins (βCD and HPβCD), in the presence or absence of a water-soluble polymer (PVP), and the anthelmintic efficacy of those complexes in naturally infected lambs.

Materials and methods

Chemicals

ABZ was acquired from Sigma-Aldrich (São Paulo, Brazil). Drugs used to develop the complexation: CDs (βCD and HPβCD) were donated from Labonathus Health Solutions (São Paulo, Brazil) and PVP was acquired from BASF (São Paulo, Brazil).

Experimental design

This work was divided in two parts: the first part involved the preparation of formulations, in vitro solubility, and dissolution assay. The second part involved the in vivo anthelmintic efficacy test of the formulations using animals naturally infected with gastrointestinal parasites.

Preparation of ABZ formulations

For the preparation of binary complexes (drug-CD), equimolar amounts of ABZ and CDs (β-cyclodextrin, βCD, and hydroxypropyl-β-cyclodextrin) were weighed and dissolved separately in purified water. For the multicomponent complexes [(drug-CD) n-polymer], equimolar amounts of ABZ and CDs (βCD or HPβCD) were weighed and dissolved separately in a 0.1% (w/v) PVP. The solutions obtained were subjected to homogenization on a magnetic stirrer (DragonLab mod. MS-MS10) for 2 h, transferred to 1000-mL, round-bottom borosilicate flasks, frozen at −78 °C, protected from light, and lyophilized (Liostop mod. L101) for 120 h. After freeze-drying, the complexes were ground in porcelain mortars and pulverized in a stainless steel sieve of 0.71 mm opening (#25 mesh, USA Standard Testing Sieve).
Solubility assay

The assay was performed by the equilibrium (Higuchi and Connors 1965): Binary complexes (ABZ/βCD or ABZ/HPβCD) and multicomponent complexes (ABZ/βCD-PVP or ABZ/HPβCD-PVP) were placed in amber bottles containing 10 mL of purified water and placed on a magnetic stirrer (DragonLab® mod. MS-MS10) for 72 h at room temperature. Aliquots of the supernatant were filtered through a 0.45-μm membrane, diluted with mobile phase (1:10), transferred to a 1.5-mL amber vial, and the concentration of dissolved drug was determined using a Prominance HPLC System (Shimadzu Corporation, São Paulo, Brazil) with a mobile phase composed of acetonitrile/0.04 M diammonium orthophosphate, pH 7.5–7.6 (41:59, v/v), a flow rate of 1.5 mL/min (isocratic), and 20 μL injection volume, through a Kromasil® RP-C18 chromatographic column of 250 × 4.6 mm ID (100 Å, 5 μm) equilibrated at room temperature. ABZ quantification was done by UV detection at 300 nm wavelength.

Dissolution assay

The assay was performed on dissolution apparatus (Ethik Technology model 299/ATTS) using rotary disk apparatus (Wood Apparatus, USA), purified water as dissolution medium, stirring speed of 100 rpm, and volume of 900 mL. ABZ and inclusion complexes (100 mg) were weighed and transferred to forms (rotary discs). A hydraulic press of 10 t (Flowscience Brazil) compacted the mixture at 250 psi for 30 s. Then, the compacted drugs were coupled to the Wood apparatus and placed in the dissolution apparatus. The assays were performed in triplicate at the intervals of 5, 10, 15, 20, 30, 40, 50, 60, 90, 120, 180, and 240 min. The amount of drug dissolved was determined using liquid chromatography, as described above. Concentrations were determined considering the aliquots volume used (Aronson 1993). The intrinsic dissolution rate was obtained from the linear regression of the ascending segment of the ratio of the accumulated amount of drug dissolved by the compacted surface area (0.5 cm²) as a function of time (Fig. 1).

Experimental animals

Forty-eight male lambs, Morada Nova breed, approximately 1-year-old, of 20 kg each, and naturally infected with gastrointestinal parasites were confined in collective pens. Animals were fed corn silage, commercial concentrate (16% crude protein), mineral salt, and water ad libitum offered in automatic drinking fountains. Animal procedures and management protocols were approved by the Ethics Committee on Animal Use (CEUA) of the Instituto de Zootecnia (IZ/APTA/SAA) and received protocol number IZ/229-16.

Efficacy test

Animals were distributed equally in six treatments groups according to their fecal egg count (FEC) and hematocrit. Fecal samples were collected directly from the rectum, and egg counting was performed as described by Ueno and Gonçalves (1998). Blood samples were collected from the jugular vein in vacuum tubes containing the anticoagulant dipotassium ethylene diamine tetra acetate hydrate (EDTA) and centrifuged (Carvalho 1999).

Ten minutes before applying the treatments, the drugs (ABZ and ABZ + complexes) were weighed on a precision scale, mixed with purified water, and shaken vigorously in amber glass vials.

The solutions were prepared according to the concentration of pure ABZ contained in each formulation. Each 5.3 g of ABZ/βCD or ABZ/βCD-PVP contained 1 g of pure ABZ, while 6.8 g of ABZ/HPβCD or ABZ/HPβCD-PVP contained 1 g of pure ABZ. The final concentration of all solution was 10 mg ABZ/mL.

Formulations were administered at a dose of 10 mg ABZ/kg of body weight, delivered orally with a syringe, animals with heads up, every 24 h for 3 days, in the afternoon (no fasting). Animals were weighed for exact dose administration of drugs according to treatments:

T1—Control (purified water)
T2—ABZ (albendazole diluted in purified water)
T3—ABZ/βCD (ABZ/βCD diluted in purified water)
T4—ABZ/βCD-PVP (ABZ/βCD-PVP diluted in purified water)
T5—ABZ/HPβCD (ABZ/HPβCD diluted in purified water)
T6—ABZ/HPβCD-PVP (ABZ/HPβCD-PVP diluted in purified water)
Fecal egg count was performed before and after treatments, and the efficacy after treatments was calculated for each animal. The efficacy was calculated using the fecal egg count reduction test (FECRT) with the following formula: FECRT\% = 100 (1 - final FEC / initial FEC), where FECRT is expressed in percentage, initial FEC (before treatment), and final FEC (10 days after the last administered dose).

Coprocultures were prepared from a pool of feces from each experimental treatment in order to identify nematode genera and determine the percentage of each genus as described by Ueno and Gonçalves (1998).

Statistical analysis

Data on FEC and FECRT were analyzed by variance analysis. Before analysis, FEC data were transformed to log (x + 1). All statistical analysis was performed using PROC GLM in SAS. Comparison between groups was performed by analysis of variance (ANOVA) followed by Tukey’s test. Differences were considered significant at $P < 0.05$.

Results

Chemicals, solubility, and dissolution assay

ABZ and ABZ + complexes prepared for this experiment were evaluated by solubility and intrinsic dissolution assay (Table 1; Fig. 1). The complexes obtained with HPβCD had higher solubility and intrinsic dissolution rate (IDR). The addition of PVP (water-soluble polymer) in the binary complexes (ABZ/βCD and ABZ/HPβCD) increased solubility and intrinsic dissolution rate (IDR).

Efficacy test

The administration of ABZ and ABZ + complexes at the dosage of 10 mg/kg body weight (based on ABZ) reduced the FEC of all treated groups ($P < 0.001$) when compared to the control group. There were no differences between T2, T3, T4, and T5 ($P > 0.05$) (Table 2). However, the percentage of efficacy was lower than 95%, considered as effective (Coles et al. 1992). Animals treated with ABZ had 70.33%, ABZ/βCD (85.33%) and ABZ/βCD-PVP (85.33%). The efficacy from complexes ABZ/HPβCD (78.37%) and ABZ/HPβCD-PVP (43.79%) was lower than expected as in vitro tests both presented high solubility and dissolution index.

In order to evaluate the efficacy of drugs over each parasitic genus, coprocultures were done with feces collected before and after treatments (Table 3). In all treated groups and in the control group, the predominance of *Haemonchus* sp. larvae was observed, followed by *Trichostongyulus* sp., *Cooperia* sp., and, to a lesser extent, the species *Oesophagostomum* sp. and *Strongyloides* sp. No difference was observed after treatments related to the predominance of *Haemonchus* in coprocultures from different treatments.

Discussion

Benzimidazoles have a broad spectrum of activity against gastrointestinal helminths; however, the low solubility of this class of substances and the inappropriate use led to therapeutic failures. The lack of management strategies, the insufficient knowledge of pharmacological features, and the insufficient understanding of relationship between pharmacological properties and host-related factors are the main causes of inefficiency and the appearance of resistance (Lanusse et al. 2014). Due to the difficulties in the developing new anthelmintic molecules, the optimization of existing compounds and strategies of use are priorities.

The efficacy of oral anthelmintic drugs is conditioned by their dissolution in the gastrointestinal tract and by its permanence at the site of action (Charikoffuki et al. 2009). The solubility of ABZ can be modified by the inclusion of CD and of water-soluble polymers such as PVP. Garcia et al. (2014) and Moriwaki et al. (2008) also evaluated ABZ combined with βCD and found excellent dissolution rates and highest level of drug release after 20 min in vitro. In our study, the inclusion of HPβCD resulted in better solubility and dissolution in comparison to βCD. HPβCD increased dissolution and drug release in less than 20 min in vitro.

Soares-Sobrinho et al. (2012) have developed and characterized inclusion complexes in binary systems including benzimidazole and randomly methylated β-cyclodextrins (RMβCD) and also in ternary systems including benzimidazoles, RMβCD, and the hydrophilic polymers hydroxypropylmethylcellulose (similar to PVP). The results showed that the binary systems had a large increase in dissolution rate ($Q > 80\%$). However, the ternary systems containing 0.1% of hydroxypropylmethylcellulose had no advantage compared to the binary inclusion complexes in a solid state.

Many in vivo tests that evaluated the dissolution and bioavailability of ABZ and complexes concluded that dissolution, bioavailability, and maximum concentration of the drug in plasma were increased with ABZ + inclusion complexes when compared to pure ABZ. Ehteda et al. (2012) evaluated the rate of dissolution and bioavailability of ABZ and βCD in vivo (mice). The dissolution rate and plasma concentration of inclusion complexes (ABZ/βC) were higher when compared to ABZ. Castillo et al. (1999) also evaluated the bioavailability of ABZ/HPβCD and active metabolite (albendazole sulfoxide) after oral ingestion in Swiss mice. The authors concluded that ABZ/HPβCD
increased the dissolution profile and decreased the time to reach the maximum plasma concentration of active metabolite when compared to ABZ alone.

Evand et al. (2002) compared the bioavailability of a solution containing the inclusion complexes (ABZ/HPβCD) with a commercial solution of ABZ (Valbanzen 1.9%®) in vivo (with sheep). Inclusion complexes had better oral bioavailability (AUC = 36.7 ± 3.4 μg h/mL) when compared to Valbanzen alone (AUC = 26.7 ± 7.9 μg h/mL), both administered at the same concentration (5 mg/kg body weight). However, the authors did not evaluate their anthelmintic efficacy. The activity of an anthelmintic drug is dependent not only on binding to receptor, but also on the ability to reach high and constant concentrations and at the therapeutic range (Alvarez et al. 2007). In our study with infected sheep, ABZ was administered at 10 mg/kg for three consecutive days. ABZ has low aqueous solubility and high permeability. Consequently, this compound requires high doses or multiple doses (or both) in order to achieve therapeutic concentrations and acceptable anthelmintic efficacy. In humans, infection for the *Echinococcus granulosus*, although ABZ is an effective anthelmintic, only about one third of patients presented complete remission or cure; 30–50% presented evidence of therapeutic response, and 20–40% of those human patients did not respond to medical management with ABZ (Pensel et al. 2014).

The complexing efficiency of the CDs with drugs can be enhanced by the addition of a small amount of a water-soluble polymer. This multicomponent complex, in general, presents greater stability and solubility than a binary complex (Lofsson et al. 2005; Lofsson 2007; Ashbahr et al. 2009). The effect of polyvinylpyrrolidone (PVP) combined with β-cyclodextrin was evaluated for the dissolution rate, oral bioavailability, and cysticidal efficacy of ABZ commercial suspension as Zentel® suspension 40 mg (GlaxoSmithKline, Mexico). The ABZ/βCD-PVP complexes exhibited the highest dissolution rate (78.5%), and its bioavailability was also significantly increased (2.3-fold). In addition, the cysticidal activity (Taenia crassiceps cysts) of ABZ/βCD-PVP complexes (83%) was greater than a commercial suspension (38%). The results suggest that the multicomponent system (ABZ/βCD-PVP) can be an alternative for the treatment of systemic helminthic diseases (Palomares-Alonso et al. 2010).

In the present work, ABZ efficacy was 70.33%. The inclusion of βCD to ABZ (ABZ/βCD) increased efficacy to 82.86% and ABZ/HPβCD increased to 78.37%. However, no differences were found between complexation and none of them reached 95% efficacy required to be an effective drug (Coles et al. 1992). The addition of PVP increased ABZ/βCD and ABZ/HPβCD solubility (Table 1). However, the anthelmintic activity decreased when PVP was added (Table 3). This hydrophile polymer has been widely used as a carrier because of low cost, high solubility, biocompatibility, and because it is considered as a pharmaceutical excipient (Sullivan et al. 2010; Santos et al. 2011). Pharmaceutical excipients are safe substances that are included in formulas to promote availability without negative interferences with the molecule of the active ingredient. PVP is stable at room temperature and also in aqueous solutions (Villanova and Sá 2009; Narang and Boddu 2015). Toxicological studies support the lack of biological activity of PVP (Schwarz 1990).

Authors such as Zhang et al. (2006) and Martínez-Marcos et al. (2016) showed a high increase in drug dissolution profile compared to pure ABZ. Zhang et al. (2006) added nanoparticles to ABZ in tests with rats and found that the drug’s absorption capacity increased when the polymer PVP was added.

The inclusion of PVP resulted in lower efficiency rates compared to PVP-free complexation (ABZ/βCD was 85.33% and ABZ/HPβCD-PVP was 82.86%). Other complexation tested was ABZ/HPβCD with 78.37% efficacy and ABZ/HPβCD-PVP with 43.79%. As long as the solubility and dissolution increased, the efficacy decreased. This numerical difference found in the efficacy test could be explained. One possible explanation is that PVP inclusion may reduce the mobility of cyclodextrins in aqueous solution leading to stability of the complexes formed (Ashbahr et al. 2009). This fact may explain the low efficacy of
formulations containing PVP. Another possibility for this numerical decrease in efficacy of HPβCD-PVP involves its high solubility and therefore the rapid biotransformation of ABZ into its inactive metabolite ABZ sulfone (ABZSO₂) (Moreno et al. 2004). When absorbed, ABZ is biotransformed into the active metabolite (ABZSO), which can be then converted to its inactive form—ABZSO₂ by liver enzymes (Delatour et al. 1986). The efficacy of BZD compounds relies on the time that the parasite is exposed to “toxic” concentrations, and the anthelmintic activity is influenced by the residence time of the drug in the animal's body (Moreno et al. 2004). According to Molento (2004), in the case of ruminants, biotransformation is reduced because ABZ adheres to the fibers present in the diet. This adhesion also occurs in commercial anthelmintics containing ABZ because an oil carrier added to the formulation improves solubilization (Ali and Hennessy 1995). In this work, the solubilization of ABZ or ABZ + complexes was evaluated in water due to the addition of CD, and the anthelmintic efficacy observed for ABZ diluted in water was 70.33%.

The parasitic genera found in the gastrointestinal tract of small ruminants included *Haemonchus* spp., *Trichostrongylus* spp., *Cooperia* spp., *Strongyloides* spp., and *Oesophagostomum* spp. *Haemonchus* spp. and *Trichostrongylus* spp. have been described as the most prevalent parasites in naturally infected animals (Cardia et al. 2011). ABZ or its combination with CD or PVP did not affect the prevalence of parasites found in coprocultures.

### Conclusion

CD (βCD and HPβCD) increased the solubility and intrinsic dissolution rate of ABZ, both favored in the presence of the water-soluble polymer. However, the increase in the solubility of ABZ did not correlate with an increase in its efficacy.

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**Compliance with ethical standards** Animal procedures and management protocols were approved by the Ethics Committee on Animal Use (CEUA) of the Instituto de Zootecnia (IZ/AFTA/SAA) and received protocol number I2/229-16.

**Conflict of interest** The authors declare that they have no conflict of interest.

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