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Research Paper

A nanocrystal-based formulation improves the pharmacokinetic performance and therapeutic response of albendazole in dogs

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Keywords
albendazole; ancylostomiasis; dogs; nanocrystals; pharmacokinetics

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Abstract

Objectives Here, we aimed to assess the pharmacokinetic performance and therapeutic response (anthelmintic efficacy) of an albendazole (ABZ) nano-sized formulation in dogs.

Methods In the pharmacokinetic study, ABZ self-dispersible nanocrystals (SDNCS) and a control formulation were administered orally to healthy dogs ($n = 6$). The concentrations of the sulphoxide metabolite in plasma were determined by high-performance liquid chromatography. For the anthelmintic efficacy trial, SDNCS and a commercially available formulation of ABZ were given to naturally parasitised dogs. The number of *Ancylostoma caninum* eggs in the faeces was determined using the McMaster technique.

Key findings The area under the curve, *T*max and *C*max for the SDNCS were improved compared to the control. The efficacy study showed no statistical differences between the SDNCS and the commercial formulation at the doses of 25 and 12.5 mg/kg. However, significant differences ($P < 0.05$) between the treatments were found at 6.25 mg/kg (a quarter of the reference dose) with a reduction in the faecal nematode egg counts of 62.0 ± 21.1% and 100 ± 0% for the control and SDNCS, respectively.

Conclusions The improved pharmacokinetic performance observed for the novel formulation of ABZ correlated with an improved *in vivo* therapeutic response against a model intestinal nematode parasite in dogs.

Introduction

The treatment and control of internal parasitic diseases in pets are relevant in terms of clinical practice as animals' growth and development might be severely affected. The canine hookworm, *Ancylostoma caninum*, is a pathogenic species of nematodes found in adult dogs and is able to cause significant anaemia, metabolic disturbance and hyponatremia in a severe infestation.[1] In addition, *A. caninum* is able to produce zoonosis presenting a risk to health in humans.[2] The high prevalence and potential public health impact of ancylostomiasis, as well as the development of resistance of *A. caninum* to the most used antiparasitic agents,[3] mean that new strategies are needed to enhance efficiency in its treatment.

ABZ, methyl [5-(propylthio)-1-H-benzimidazol-2yl] carbamate, a benzimidazole with a broad-spectrum antiparasitic effect, acts by preventing the polymerisation of microtubules in eukaryotic cells.[4] The high efficacy shown by ABZ is compromised due to its low aqueous solubility, which leads to erratic bioavailability and therapeutic failures.[5] ABZ is a class II drug in the Biopharmaceutics Classification System,[6] which means it exhibits low solubility and high permeability. For class II drugs, an improvement in the solubility might result in increased drug absorption.

To address this, multiple approaches have been aimed at improving ABZ water solubility and dissolution rates, such as the formulation chitosan microspheres,[7] oil/water emulsions,[8] microcrystals,[9] solid dispersions,[10] incorporation into liposomes,[11] complexation with cyclodextrins[12] and co-grinding.[13] Moreover, multiple authors have demonstrated that enhanced bioavailability of the parent drug/active metabolite is correlated with an improved antiparasitic effect.[5,7,11,12,14–16]

Nevertheless, most of the strategies described above have shown limited capacity in the improvement of ABZ bioavailability/efficacy, and the development of free-organic solvent, inexpensive, highly reproducible and industrially feasible approaches is needed. In this context, the formulation of drug nanocrystals (NC) has emerged as a promising tool for the formulation of poorly soluble drugs.

NC are defined as nanoparticles composed of practically 100% drug, being generally stabilised by surfactants or polymeric steric agents and with a mean particle size is below 1 μm.[17] NC are generally produced as aqueous dispersions (nanosuspensions), which usually need further solvent removal to obtain re-dispersible powders.[18] NC display a series of benefits in oral administration of poorly soluble drugs, including improved absorption, higher bioavailability, rapid action onset, reduced fed/fasted state and intersubject variability.[19]

In a previous work, we demonstrated that ABZ formulated as powdered self-dispersible NC (SDNCS) presented a high redispersion capacity, as well as enhanced saturation concentration and dissolution rate.[20] However, the *in vivo* performance of this formulation remained unexplored. To address this, here, we aimed to assess the pharmacokinetic performance of ABZ SDNCS in healthy dogs. The therapeutic response of this novel formulation in dogs naturally parasitised with the nematode *A. caninum* was also evaluated. Given the lack of reports on the pharmacokinetic characterisation of ABZ nano-sized formulations and complementary *in vivo* efficacy trials, this work presents a great novelty.

Materials and Methods

Chemicals

Pharmaceutical grade ABZ was purchased from Todo Droga®, Córdoba, Argentina, and Poloxamer 188 (P188) was from Rumapel, a representative of BASF® in Argentina. All the other reagents used in this work were of pro-analysis quality, and ultrapure water (HF-Super Easy Series, Heal Force, Shanghai, China) was used in all the assays. For the anthelmintic response trial, Vermizole® (LAFEDAR, Paraná, Argentina) (VMZ), a commercially available immediate release formulation of ABZ in 200-mg grooved tablets and produced by wet granulation, was used as a control.

Preparation of ABZ Nanocrystals

SDNCS were prepared by a top-down technique, as described in previous work.[20] Briefly, nanosuspensions of ABZ and P188 (1 : 1) were prepared by 30 cycles of high-pressure homogenisation at 1200 bar (Avestin C5 EmulsiFlex®, Ottawa, ON, Canada). The nanocrystal suspension was dried on a laboratory-scale Mini Spray-dryer Büchi B-290 (Büchi Labortechnik AG, Flawil, Switzerland) equipped with a dehumidifier module. A two-fluid nozzle with a cap orifice diameter of 0.5 mm was used, and the operating conditions were as follows: atomisation air (l/h), 819; aspiration, 75%; temperature, 45 °C; and pump, 5%.

Particle size

The particle size and polydispersity index (PDI) values of the SDNCS were determined by photon correlation spectroscopy (Delsa™ Nano C Particle Analyzer, Beckman and Coulter, Inc. Brea, CA, USA). Before taking measurements, samples were diluted with ultrapure water to obtain the required absorption intensity. For this purpose, 10 mg of powder were added to 5 ml of water, and the resultant suspension was shaken by hand for 1 min before carrying out the assays.

Dissolution study

Dissolution tests of pure ABZ, a physical mixture (PM) and the nanocrystals placed in transparent hard gelatine capsules, were performed using a USP XXIV dissolution apparatus 1 (SOTAX AT 7 Smart, Westborough, MA, USA). The rotational basket speed was set at 75 rpm, and the temperature kept constant at 37 ± 0.5 °C. The assayed amount of ABZ was 50 mg in all experiments. For the dissolution medium, 900 ml of HCl 0.1 M was used. Five-millilitre aliquots were withdrawn at predetermined time intervals over 2 h with reposition of fresh medium. Samples were filtered, and the concentration of dissolved drug was measured at 299 nm using a UV–Vis spectrophotometer (Thermo Evolution® 300, Waltham, MA, USA). All dissolution assays were performed in triplicate.

Physical mixture

For the dissolution and pharmacokinetic studies, a PM was used as a control. This was prepared in an agate mortar by blending softly ABZ and P188 powders in a 1 : 1 ratio for 10 min.

Pharmacokinetic Study

Experimental setup

Six healthy (3–6-year-old) and free parasite cross-breed dogs (four males and two non-pregnant females), weighing 18.3 ± 5.2 kg, were used in this trial. Animals were fed 12 h before the treatment and refed 12 h after treatment. Experimental dogs were randomly allocated into two groups (n = 3) (group I: animals #1, #2, #3; group II: animals #4, #5, #6), which received two different treatments using a crossover design. Each experimental treatment was given orally to the six animals in two phases. In Phase I, group I received SDNCS (treatment A), and animals in group II received the PM (treatment B). After 21 days of a wash-out period, both treatments were reversed and repeated as Phase II. ABZ was administered in a single dose of 25 mg/kg in both treatments.

Blood samples were collected from the antebrachial vein using a 18-G catheter before administration (time 0) and at 0.25, 0.5 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 16 and 24 h postadministration of the respective formulations. Blood samples were immediately transferred into heparinised tubes and centrifuged at 2000*g* for 15 min; the recovered plasma was stored at −20 °C until analysis by HPLC.

Analysis of ABZ and its sulphoxide metabolite

The quantification of the ABZ and its sulphoxide metabolite isolated from plasma was performed by an HPLC methodology described in a previous work.[21] The concentration vs time curve for the metabolite ABZSO in plasma for each individual animal after the different treatments was fitted using PK Solution 2.0 (Summit research services, Ashland, OH, USA). The Peak concentration (C_{max}) and time to peak concentration (T_{max}) were obtained from the plotted concentration–time curve of each analyte. The area under the concentration–time curve (AUC) was calculated by the linear trapezoidal rule.[22]

Anthelmintic efficacy study

Animals

Faecal samples of a total of 96 cross-breed dogs (all sterilised) from a canine shelter of Montevideo, Uruguay, were collected. Animals were allocated into individual roofed separate kennels with a concrete/grass floor and fed once daily. With the aim to identify the naturally parasitised dogs, all the collected samples were analysed using a Willis flotation technique.[23] Based on a detailed analysis of eggs morphology,[24] we identified 42 dogs infected with *A. caninum* that were recruited for this trial. Of these, seven were co-infected with *Trichuris vulpis* (75 ± 45 eggs per gram (EPG)). However, due to the low prevalence of *T. vulpis*, *A. caninum* was chosen as the model nematode to evaluate the therapeutic response of our novel formulation. The initial egg count (day 0) for *A. caninum* was 640 EPG (n = 42), ranging between 80 and 1240 EPG. The average weight of the recruited animals (22 males and 20 females) was 25.0 ± 5.5 kg.

Experimental design

The naturally parasitised animals were randomly allocated into seven groups (n = 6). Groups I, II and III received ABZ SDNCS at 6.25, 12.5 and 25 mg/kg per day, respectively, during 3 days. Groups IV, V and VI were treated with VMZ following the same therapeutic scheme of groups I to III. Group VII remained untreated until the trial was finished.

Faecal samples of each naturally parasitised dog were collected at day 0 (considered as reference) and at days 1, 2, 3, 7, 14 and 30 after the last ABZ administration. A Willis flotation technique and a modified McMaster faecal egg counting procedure[25] were performed on each sample.

The faecal egg count reduction (FECR) on each animal was determined by the following equation:

$$\mathrm{FECR} = \frac{\mathrm{EPG\,(day\,0)} - \mathrm{EPG\,(day}\,n\,\mathrm{post-treatment)}}{\mathrm{EPG\,(day\,0)}} \times 100 \quad (1)$$

Statistical analysis

In the pharmacokinetic study, a non-parametric Mann–Whitney test was used for the multiple statistical comparisons of the data obtained from the different groups. For the anthelmintic efficacy assay, a paired sample t-test and Kruskal–Wallis test (post hoc: Mann–Whitney test) were used for the comparison of the SDNCS and VMZ treatments. These statistical analyses were performed using the Info Stat software.[26] For all statistical comparisons, a p value less than 0.05 ($P < 0.05$) was considered significant.

Ethical considerations

Animal procedures and management protocols were approved by the Ethics Committee according to the Animal Welfare Policy of the Faculty of Veterinary, Universidad de la República, Montevideo, Uruguay (www.fvet.edu.uy). Animals were routinely observed by clinically experienced staff to report any adverse reaction during both trials.

Results and Discussion

Nanocrystal formulation

Although the experimental formulation of ABZ (SDNCS) and its characterisation was developed in detail in our previous report,[20] the particle size and PDI of the batch used in this work were tested, being 415.69 ± 7.40 nm and 0.245 ± 0.006, respectively.

The dissolution behaviour of the formulation was also studied. We found that the SDNCS presented a high dissolution rate; the amount of ABZ dissolved at 1 h was 74.43%, while the respective value for the PM and pure ABZ at 1 h were 7.77% and 3.7% (Figure 1). In agreement with the Noyes–Whitney, and Ostwald–Freundlich equations, a decrease in particle size led to an increase in the specific contact surface, enhancing the drug dissolution velocity and saturation solubility.[27,28] The high percentage of P188 in the formulation (50%) permits the formation of water-soluble links between the nano-sized drug particles, thus resulting in a complete redispersion of the powdered material, which was visually confirmed as the dissolution vessels were completely opalescent after the disintegration of the transparent gelatin capsules within 10 min of the test. The potential surfactant effect of P188 in the dissolution rate of ABZ is negligible. This can be confirmed analysing Figure 1 where pure ABZ and the PM (containing 50% of P188) presented similar dissolution profiles.

On the other hand, amorphisation of ABZ during the formulation could lead to an increased dissolution rate; nevertheless, we have previously demonstrated that the crystalline state of the pure ABZ drug remains unchanged in the SDNCS.[20]

The observed higher dissolution rate does not necessarily guarantee an improvement in pharmacokinetic parameters, especially in small animals with a short gastrointestinal tract (GIT) and rapid intestinal transit, such as dogs.[29]

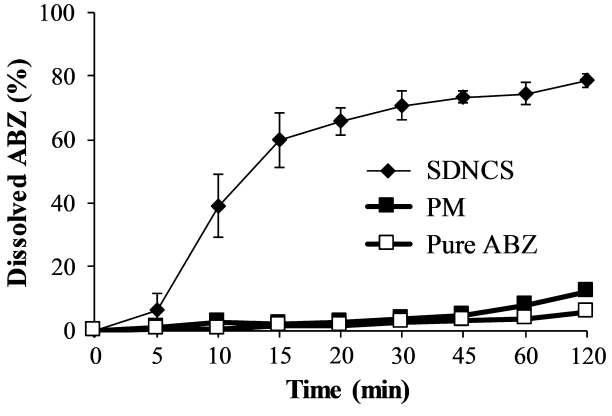

Figure 1 Dissolution profile of ABZ nanocrystals (SDNCS), PM and pure ABZ placed in transparent hard gelatin capsules in 900 ml of HCl 0.1 M, using apparatus 2 (75 rpm) at 37 ± 0.5 °C.

Pharmacokinetic study

Figure 2 shows the pharmacokinetic profiles of ABZSO after a single oral administration of SNDCS and the control formulation at 25 mg/kg. The ABZ parent drug was not detected in the plasma, whereas ABZSO was detected in both treatments (SNDCS and control), for up to 8 and 16 h, respectively. SDNCS produced a rapid increase in the sulphoxide plasma concentration, achieving a maximum at 1.5 ± 0.5 h, while the control presented the C_{max} at 3 ± 1 h. The $AUC_{0\rightarrow\infty}$ value for SDNCS (24.85 ± 7.40 μg h/ml) was 275% higher than that obtained for the control group (6.62 ± 4.30 μg h/ml). The

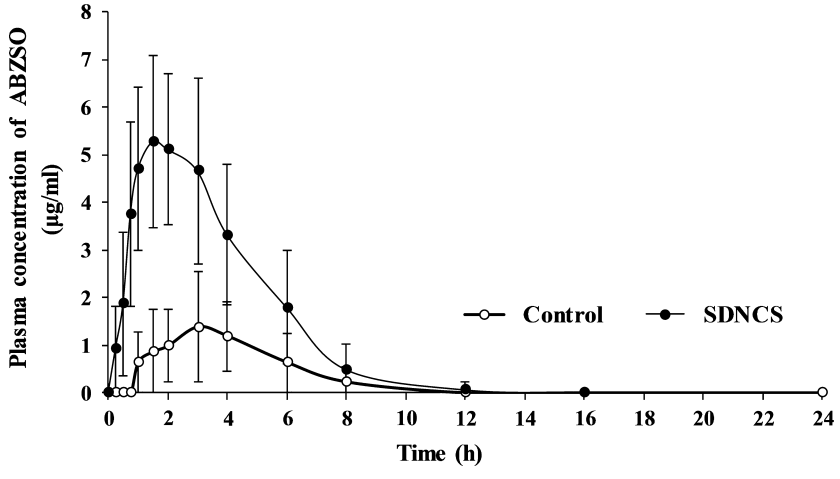

Figure 2 Plasma concentration of ABZSO after a single oral administration of the nano-sized ABZ (SDNCS) 25 mg/kg and a physical mixture containing the same composition of the experimental preparation.

C_{max} value observed for SDNCS was fivefold higher ($P < 0.05$) than the control (5.63 ± 1.74 μg/ml and 1.53 ± 1.04 μg/ml, respectively) (see Table 1).

When administered orally, ABZ is extensively metabolised to an active sulphoxide metabolite (ABZSO) in the liver, and further oxidation leads to the second inactive metabolite, albendazole sulphone.[30] To achieve a suitable plasma concentration of the ABZSO, microparticles containing the drug NC (microcomposites) must disintegrate and then, ABZ (a weak base) must dissolve in the acid environment of the stomach to finally permeate the duodenal membrane (Figure 3a–c). NC of basic drugs are more easily affected by pH variation in the GIT. For weak bases, a nano-sized drug formulation will dissolve fast and more efficiently in the low stomach pH environment, but in the transit to duodenum, the rise in pH may illicit uncontrolled precipitation of drug substance.[27] Nonetheless, this phenomenon seemed not to affect our formulation as the pharmacokinetic performance was truly increased. We hypothesise that although the GIT transit in dogs is rapid, the gastric emptying is gradual, thus permitting the absorption of the active.

Table 1 Pharmacokinetic parameters of the sulphoxide metabolite (ABZSO) after a single oral administration of ABZ SDNCS and control (PM) at 25 mg/kg

	*T*max (h)	*C*max (μg/ml)	$AUC_{0\to\infty}$ (μg h/ml)
SDNCS	1.5 ± 0.5*	5.63 ± 1.74*	24.85 ± 7.40*
Control (PM)	3.0 ± 1.0	1.53 ± 1.04	6.62 ± 4.30

$AUC_{0\to\infty}$, area under the plasma concentration–time curve up to time infinity; *C*max, peak plasma concentration; *T*max, time to reach peak plasma concentration. Data are expressed as mean ± SD ($n = 6$). *$P < 0.05$ vs control.

It is important to note that P188 has the disadvantage of forming *in situ* thermo-sensitive gels that might delay the drug release from the formulation, as reported by Dib *et al.*[31] However, according to this study, this behaviour seems not to affect drug absorption as the *in vivo* performance increased for the SDNCS compared to the control.

According to multiple reports, the absorption of benzimidazole anthelmintic drugs in cats, dogs and humans is limited by their low dissolution rates in gastric fluids.[21] Consequently, these antiparasitic agents must be administrated at high or multiple doses to provide sustained concentrations at the parasite site.[30] In this context, and based on the promising outcomes of the pharmacokinetic assay, it was propitious to move forward with an *in vivo* efficacy study testing the innovative formulation in 'real-use' conditions.

Therapeutic response assay

To evaluate the correlation between an increased systemic ABZSO availability and its capacity to reach the haematophagous adult worms in the intestine lumen (Figure 3d), an anthelmintic efficacy study was performed. For this, a commercial formulation (VMZ) and an SNDCS were orally administered to dogs naturally parasitised with *A. caninum,* and the egg output in faeces was measured. The seven animals infected with *Trichuris vulpis* showed the absence of this parasite eggs in faeces in all the post-treatment assayed days (data not shown).

Regarding *A. caninum,* we are aware that the female worm is able to modulate the egg production in response to the degree of intestinal crowding. Several reports indicate that an increase in worm burden in the intestine correlates with a decrease in the egg output, but the mechanism for this remains unknown.[3,32,33] Besides, it has been

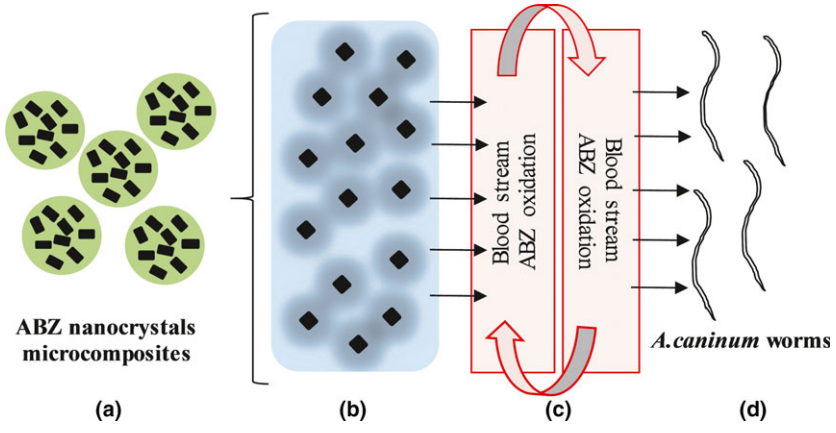

Figure 3 (a) ABZ NC in microcomposites before its administration. (b) Redispersion of SDNCS and dissolution of ABZ in the aqueous and acid conditions of the stomach. (c) Permeation of ABZ into the blood stream in the small intestine and oxidation in liver. (d) ABZ reaches the hematophagus parasite worms located in GIT lumen.

Table 2 *Ancylostoma caninum* faecal egg count reduction expressed as FECR ± SD (n = 6) in the groups treated with the control and SDNCS at three dose levels: 25, 12.5 and 6.25 mg/kg

	Treatments								
	25 mg/kg			12.5 mg/kg			6.25 mg/kg		
Day	Control	SDNCS	*P* value	Control	SDNCS	*P* value	Control	SDNCS	*P* value
1	91.0 ± 14.0	100 ± 0	0.455	100 ± 0	100 ± 0	0.999	55.5 ± 28.2	100 ± 0	**0.0022**
2	94.3 ± 9.0	100 ± 0	0.455	100 ± 0	100 ± 0	0.999	61.3 ± 23.5	100 ± 0	**0.0022**
3	96.2 ± 6.0	98.3 ± 4.1	0.727	100 ± 0	100 ± 0	0.999	62.0 ± 21.1	100 ± 0	**0.0022**
4	96.2 ± 6.0	100 ± 0	0.455	100 ± 0	100 ± 0	0.999	59.9 ± 20.9	100 ± 0	**0.0022**
15	100 ± 0	100 ± 0	0.455	100 ± 0	100 ± 0	0.999	58.9 ± 23.0	100 ± 0	**0.0022**
30	79.8 ± 10.6	90.7 ± 14.8	0.225	79.5 ± 11.5	83.6 ± 4.6	0.366	41.6 ± 27.3	100 ± 0	**0.0022**

Control, commercial formulation of ABZ Vermizole®; SDNCS, self-dispersible nanocrystals of ABZ. P value < 0.05 paired sample t-test and a Kruskal–Wallis test (post hoc: Mann–Whitney test) indicate significance.

highlighted the necessity for a critical examination of the ability of FECR in *A. caninum* drug resistance studies.[34] However, deeper studies should include animal euthanasia, seriously compromising ethical aspects of our trial.

Table 2 shows the FECR (mean ± SD) of the treated groups with three doses (once daily) of the control and SDNCS at three dose levels (25, 12.5 and 6.25 mg/kg). All of the treated groups presented a high reduction in the parasite egg count, even in the first-day post-treatment. Groups I to VI presented significant differences in the FECR when compared with the untreated group (VII) in all of the assayed days ($P < 0.05$). Furthermore, all samples were taken at days 1, 2, 3, 7, 14 and 30 presented significant differences with their respective amount of EPG at day zero ($P < 0.05$) in all of the treated groups.

At a dose of 25 mg/kg (dose of reference),[30] the reduction in the egg counting was close to 100% and no significant difference was observed between the formulations ($P > 0.05$). Similar results were obtained when the dose was reduced to 50% (12.5 mg/kg), as both treatments practically eradicated the eggs, with no statistical difference ($P > 0.05$) in the period of time evaluated. Conversely, significant differences ($P < 0.05$) between SDNCS and VMZ were observed when the administered dose was 6.25 mg/kg (a quarter of the reference dose) as the nano-sized formulation permitted a reduction of 100 ± 0% in the egg counting, while the control only produced a maximum reduction of 62.0 ± 21.1%.

Relevant results regarding the potential of our innovative formulation efficacy can be obtained from the analyses of Figure 4, where the reference treatment (VMZ 25 mg/kg) was compared to the experimental treatments (VMZ 12.5 mg/kg ($P > 0.05$) and SDNCS 6.25 mg/kg ($P > 0.05$)). The absence of statistical significance among these three groups suggests that the SDNCS form can achieve higher ABZSO concentrations at the parasite location, improving ABZ therapeutic efficacy against *A. caninum*, even when using a quarter of the recommended dose (see Figure 3d). However, a decrease to 50% in the dose for VMZ produced a similar decrease in FECR compared to the reference

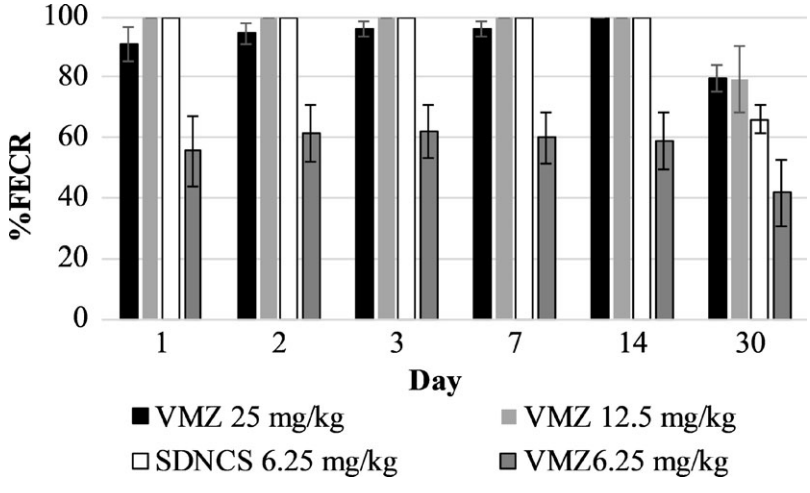

Figure 4 Comparison of FECR values between the SDNCS at 6.25 and 12.5 mg/kg and the commercial formulation (VMZ) at 25 and 12.5 mg/kg.

treatment, suggesting that overdosing is occurring in parasitic diseases. Nevertheless, further investigation in this particular point is needed.

It is worth highlighting the relevance of this potential enhancement of ABZ efficacy, particularly from a toxicological point-of-view. The potential toxicity of ABZ includes retardation of weight gain, readily reversible anaemia, slight leucopenia, hypercholesterolaemia and other non-specific and variable changes in clinical chemistry test results and slight proteinuria. Additionally, the mutagenic potential of ABZ has been recognised by the European Medicines Agency (EMA).[35] Other reports indicate bone marrow toxicosis after ABZ administration in cats and dogs.[36,37] In this case, using SDNCS, we could obtain a similar efficiency using a dose four times lower than traditional formulations currently available in the market. Thus, a consequent reduction in side effects and the cost of treatment are expectable.

Conclusions

The dissolution rate of ABZ from nanocrystals was dramatically increased compared to PM and pure drug. This was in line with the pharmacokinetic performance of nanosized ABZ in dogs. A marked improvement of ABZSO kinetic behaviour was observed for the novel test formulation (i.e. AUC values threefold higher than the control formulation being the PM).

The therapeutic response, assessed as clinical anthelmintic efficacy in dogs, showed similar egg reduction counts for the SDNCS ABZ formulation using a dose that is four times lower than the commercial formulation. These are promising results that encourage us to further optimise this formulation for human medicine as the treatment of a large number of parasitic diseases fails due to the unfavourable properties of this type of poorly water-soluble anthelmintic drugs.

Declarations

Conflict of interests

The Authors declare that they have no conflict of interests to disclose.

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References

1. Bowman DD *et al. Georgis' Parasitology for Veterinarians*, 8th edn. St. Louis: Saunders, 2003: 422.
2. Prociv P, Croese J. Human enteric infection with Ancylostoma caninum: hookworms reappraised in the light of a "new" zoonosis. *Acta Trop* 1996; 62: 23–44.
3. Kopp S *et al.* High-level pyrantel resistance in the hookworm Ancylostoma caninum. *Vet Parasitol* 2007; 143: 299–304.
4. Lacey E. Mode of action of benzimidazoles. *Parasitol Today* 1990; 6: 112–115.
5. Pensel PE *et al.* Enhanced chemoprophylactic and clinical efficacy of albendazole formulated as solid dispersions in experimental cystic echinococcosis. *Vet Parasitol* 2014; 203: 80–86.
6. Jung H *et al.* Absorption studies of albendazole and some physicochemical properties of the drug and its metabolite albendazole sulphoxide. *J Pharm Pharmacol* 1998; 50: 43–48.
7. Abulaihaiti M *et al.* Efficacy of Albendazole-Chitosan Microsphere-based Treatment for Alveolar Echinococcosis in Mice. *PLoS Negl Trop Dis* 2015; 9: e0003950. https://doi.org/10.1371/journal.pntd.0003950.
8. Mingjie W *et al.* Albendazole–soybean oil emulsion for the treatment of human cystic echinococcosis: Evaluation of bioavailability and bioequivalence. *Acta Trop* 2002; 83: 177–181.
9. Priotti J *et al.* Albendazole Microcrystal Formulations Based on Chitosan and Cellulose Derivatives : physicochemical Characterization and In Vitro Parasiticidal Activity in Trichinella spiralis Adult Worms. *AAPS PharmSciTech* 2016;. https://doi.org/10.1208/s12249-016-0659-z.
10. Castro SG *et al.* Enhanced dissolution and systemic availability of albendazole formulated as solid dispersions. *Pharm Dev Technol* 2012; 18: 1–9.
11. Wen H *et al.* Pharmacology and efficacy of liposome-entrapped albendazole in experimental secondary alveolar echinococcosis and effect of co-administration with cimetidine. *Parasitol* 1996; 113: 111–121.
12. Palomares-alonso F *et al.* Two novel ternary albendazole – cyclodextrin – polymer systems : dissolution, bioavailability and efficacy against Taenia crassiceps cysts. *Acta Trop* 2010; 113: 56–60.
13. Vogt M *et al.* Dissolution improvement of four poorly water soluble drugs by cogrinding with commonly used excipients. *Eur J Pharm Biopharm* 2008; 68: 330–337.
14. Shuhua X *et al.* Augmented bioavailability and cysticidal activity of albendazole reformulated in soybean emulsion in mice infected with Echinococcus

granulosus or Echinococcus multilocularis. *Acta Trop* 2002; 82: 77–84.
15. Ceballos L *et al.* Albendazole treatment in cystic echinococcosis: pharmacokinetics and clinical efficacy of two different aqueous formulations. *Parasitol Res* 2008; 103: 355–362.
16. Velebny S *et al.* Effect of treatment with free and liposomized albendazole on selected immunological parameters and cyst growth in mice infected with Echinococcus multilocularis. *Parasitol Int* 2004; 53: 315–325.
17. Keck CM, Müller RH. Drug nanocrystals of poorly soluble drugs produced by high pressure homogenisation. *Eur J Pharm Biopharm* 2006; 62: 3–16.
18. Müller RH *et al.* State of the art of nanocrystals – Special features, production, nanotoxicology aspects and intracellular delivery. *Eur J Pharm Biopharm* 2011; 78: 1–9.
19. Shegokar R, Müller RH. Nanocrystals: industrially feasible multifunctional formulation technology for poorly soluble actives. *Int J Pharm* 2010; 399: 129–139.
20. Paredes AJ *et al.* Self-dispersible nanocrystals of albendazole produced by high pressure homogenization and spray-drying. *Drug Dev Ind Pharm* 2016; 42: 1564–1570.
21. Castro SG *et al.* Comparative plasma exposure of albendazole after administration of rapidly disintegrating tablets in dogs. *Biomed Res Int* 2013; 920305. https://doi.org/10.1155/2013/920305.
22. Gibaldi M, Perrier D. *Pharmacokinetics*. 2nd. New York: Marcel Dekker Inc., 1982.
23. Willis HA. Simple Levitation Method for the Detection of Hookworm Ova. *Med J Aust* 1921; 2: 375–376.
24. Thienpont D *et al. Diagnosing helminthiasis through coprological examination*. 2nd ed. Beerse, Belgium: Janssen Research Foundation, 1986. 187 pp.
25. Dib A *et al*. Antiparasitic efficacy of a Ricobendazole controlled release formulation against Ancylostoma caninum and Trichuris sp intended for oral administration in dogs. *Veterinaria (Montevideo)* 2016; 52: 11–21.
26. Di Rienzo JA *et al. InfoStat version 24–03-2016*. Córdoba, Argentina: Universidad Nacional de Córdoba, 2016.
27. Gao L *et al.* Drug nanocrystals: in vivo performances. *J Control Release* 2012; 160: 418–430.
28. Mauludin R *et al.* Development of an oral rutin nanocrystal formulation. *Int J Pharm* 2009; 370: 202–209.
29. Sánchez SF *et al.* Comparative Availability of Two Oral Dosage Forms of Albendazole in Dogs. *Vet J* 2000; 160: 153–156.
30. Jones DG *et al.* Pharmacological approaches towards rationalizing the use of endoparasitic drugs in small animals. *J Vet Pharmacol Ther* 2006; 29: 443–457.
31. Dib A *et al.* Albendazole sulphoxide kinetic disposition after treatment with different formulations in dogs. *J Vet Pharmacol Ther* 2011; 34: 136–141.
32. Krupp IM. Effects of crowding and of superinfection on habitat selection and egg production in Ancylostoma caninum. *J Parasitol* 1961; 47: 957–961.
33. Sarles MP. The effect of age and size of infection on the egg production of the dog hookworm, Ancylostoma caninum. *Am J Hyg* 1929; 10: 658.
34. Kopp S *et al.* Application of in vitro anthelmintic sensitivity assays to canine parasitology: detecting resistance to pyrantel in Ancylostoma caninum. *Vet Parasitol* 2008; 152: 284–293.
35. Dayan AD. Albendazole, mebendazole and praziquantel. Review of non-clinical toxicity and pharmacokinetics. *Acta Trop* 2003; 86: 141–159.
36. Stokol T *et al.* Development of bone marrow toxicosis after albendazole administration in a dog and cat. *J Am Vet Med Assoc* 1997; 210: 1753–6.
37. Meyer EK. Adverse Events Associated With Albendazole and Other Products Used for Treatment of Giardiasis in Dogs. *J Am Vet Med Assoc* 1998; 213: 44–6.