Preliminary Communication

Therapeutic Delivery

Albendazole nanocrystals with improved pharmacokinetic performance in mice

Alejandro J Paredes[1], Sergio Sánchez Bruni[2], Daniel Allemandi[1], Carlos Lanusse[2] & Santiago D Palma*,[1]

[1]Unidad de Investigación y Desarrollo en Tecnología Farmacéutica (UNITEFA), CONICET & Departamento de Ciencias Farmacéuticas, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba. Ciudad Universitaria, X5000HUA-Córdoba, Argentina

[2]Laboratorio de Farmacología, Centro de Investigación Veterinaria de Tandil (CIVETAN), UNCPBA-CICPBA-CONICET, Facultad de Ciencias Veterinarias, UNCPBA, Tandil, Argentina

* Author for correspondence: Tel./Fax: +540 351 535 3865 int: 53363; sdpalma@fcq.unc.edu.ar

Aim: Albendazole (ABZ) is a broad-spectrum antiparasitic agent with poor aqueous solubility, which leads to poor/erratic bioavailability and therapeutic failures. Here, we aimed to produce a novel formulation of ABZ nanocrystals (ABZNC) and assess its pharmacokinetic performance in mice. **Results/methodology:** ABZNC were prepared by high-pressure homogenization and spray-drying processes. Redispersion capacity and solid yield were measured in order to obtain an optimized product. The final particle size was 415.69±7.40 nm and the solid yield was 72.32%. The pharmacokinetic parameters obtained in a mice model for ABZNC were enhanced ($p < 0.05$) with respect to the control formulation. **Conclusion:** ABZNC with improved pharmacokinetic behavior were produced by a simple, inexpensive and potentially scalable methodology.

First draft submitted: 4 September 2017; Accepted for publication: 23 November 2017; Published online: 12 January 2018

Keywords: albendazole • high-pressure homogenization • mice • nanocrystals • nanoparticles • pharmacokinetics • spray drying

The design and production of drug nanocrystals (NC) has been treated as the ultimate universal formulation strategy of drugs with poor solubility and high permeability [1]. NC by definition are carrier-free nanoparticulate systems composed of 100% drug, being generally stabilized by surfactants or polymeric steric agents; according to the definition of nanoparticles, the mean particle size is below 1 µm [2]. NC are generally produced as aqueous dispersions (nanosuspensions), which usually need further solvent removal in order to obtain redispersible powders [3]. NC display a series of benefits in the oral administration of poorly soluble drugs, including improved absorption, higher bioavailability, rapid action onset, reduced fed/fasted state and intersubject variability [4]. The outstanding features of NC have caught the attention of the industry and a total of six products have been launched on the market [5].

Albendazole (ABZ) is a methyl carbamate benzimidazole (BZD) antiparasitic agent with a broad-spectrum activity that acts by preventing tubulin aggregation [6]. The high efficacy of ABZ is compromised by its extremely low aqueous solubility, which has been related to a poor/erratic bioavailability and therapeutic failures [7]. ABZ is a class II drug in the Biopharmaceutic Classification System [8], which means it exhibits low solubility and high permeability. For class II drugs, an improvement in 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 preparation of chitosan-microspheres [9], oil/water emulsion [10], microcrystals [11], solid dispersions [12], incorporation into liposomes [13], complexation with cyclodextrins [14], co-grinding [15] and more recently, several nanoparticulate formulations [16–19]. Moreover, multiple authors have demonstrated that the enhanced bioavailability of the parent drug/active metabolite is correlated with an improved *in vitro–in vivo* efficacy [7,9–11,13,14,16–19].

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.

newlands press

We have recently reported a methodology for the preparation of an ABZ nano-sized redispersible powder (ABZNC) by the combination of high-pressure homogenization (HPH) and spray drying (SD), besides we performed the *in vitro* characterization of the obtained materials [20]. Nevertheless, an optimization of this process and the pharmacokinetic (PK) performance of the ABZNC remained unexplored. Then, the aim of this work was to study the influence of different parameters involved in the ABZNC production technique and to assay the pharmacokinetic performance of this novel formulation in mice.

Materials & methodology

Chemicals

ABZ pharmaceutical grade was purchased from Todo Droga®, Córdoba, Argentina and Poloxamer 188 (P188) was provided from Rumapel, a representative of BASF® in Argentina. All other reagents used in this work were of proanalysis quality, and ultrapure water (HF-Super Easy Series, Heal Force, Shanghai, China) was used in all assays.

Production of ABZ nanosuspensions

ABZ nanosuspensions were prepared as follows: 2.5 g of ABZ and 1.25 or 2.5 g of P188 (Suspension 1 [S1] and Suspension 2 [S2], respectively] were accurately weighed and ground in a mortar; deionized water was gradually added until 100 g of a homogeneous suspension was obtained. Afterwards, the samples were transferred to a beaker and sonicated for 5 min. A premilling process of three cycles at 500 bar and 30 cycles of HPH at 1200 bar (Avestin C5 Emulsiflex®, Ottawa, Ontario, Canada) was applied. The temperature was kept at 10°C using a heat exchanger provided by the homogenizer manufacturer.

Nanocrystals production by spray-drying

S1 and S2 were 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: atomization air (l/h): 819, aspiration: 75%, temperature (°C): 45 and pump: 5%. S1 was also dried with the addition of 1.25 g of the supporting agents, anhydrous lactose (L) and colloidal silicon dioxide (CSD), 10 min before drying, under magnetic stirring. Different L-CSD percent ratios were used in this experiment, namely 100–0, 75–25, 60–40 and 50–50. In order to maintain sample homogeneity, slow magnetic stirring was kept constant during the drying process. The powders obtained by SD were evaluated by measuring particle size, polydispersity index (PDI), solid yield (SY) and moisture content (MC). The recovered SY was calculated as the ratio of the powder weight collected after each drying experiment (Wf, dry basis) and the initial amount of components in the prepared suspensions (except water) (Wi, dry basis), as indicated in Equation 1.

$$SY = \frac{Wf}{Wi} x\,100 \qquad \text{(Equation 1)}$$

Particle size & PDI

The particle size and PDI values of the ABZNC 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 in order 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 during 1 min before carrying out the assays.

Moisture content

The powder MC was measured immediately after the SD step in a moisture analyzer with halogen heating (OHAUS M45®, OHAUS, Greifensee, Switzerland).

Dissolution study

50 mg of pure ABZ and the equivalent amount of the active in a physical mixture (PM) and ABZNC were placed in transparent gelatin capsules and assayed using a USP XXIV dissolution apparatus 1 (Sotax AT 7 Smart, Sotax, MA, USA) at 37 ± 0.5°C. The dissolution media was HCl 0.1 M (900 ml) and the basket speed was set at 75 rpm. 5 ml samples were taken at predetermined time intervals over 2 h with reposition of fresh medium. The concentrations of

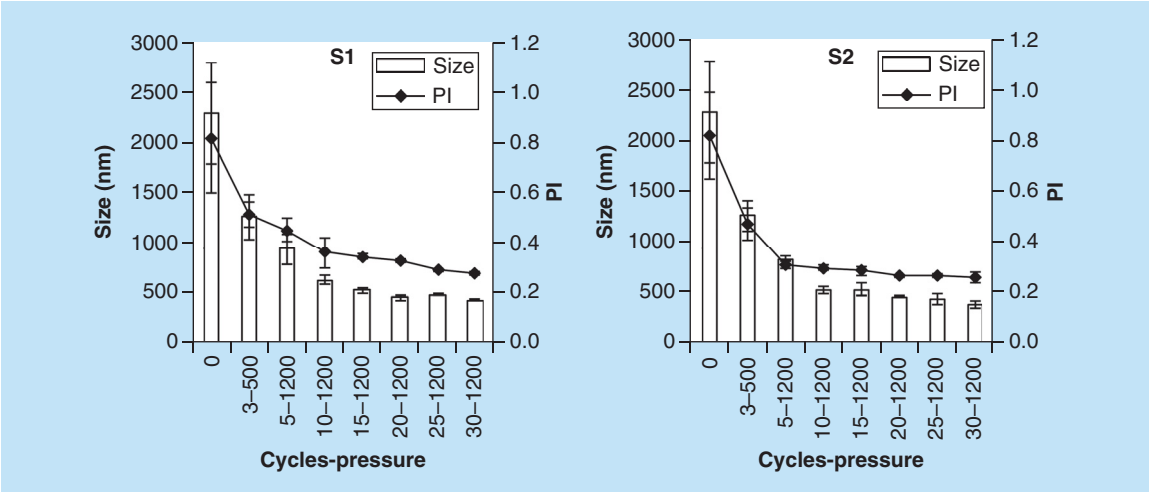

Figure 1. Particle size and polydispersity index (PI) reduction of S1 and S2 containing albendazole and P188 at different ratios (1:0.5 and 1:1 respectively). Processed by high-pressure homogenization at 30 cycles at 1200 bar.

dissolved ABZ in the aliquots were determined using a UV–Vis spectrophotometer (Evolution® 300, Thermofisher Scientific, MA, USA) at 297 nm. The dissolution rates of all samples were measured 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 grounding softly ABZ and P188 powders in a 1:1 ratio for 10 min.

Pharmacokinetic assay

The PK study was performed in male Balb/c mice. The animals were housed in a temperature controlled (21 ± 2°C), light-cycle (12 h light/dark cycle) room with food and water provided *ad libitum.* 112 healthy mice were allocated into two groups of 56 animals and treated with ABZNC and the control (PM), respectively. Both treatments were suspended in ultrapure water and manually shaken before each administration. A single dose of ABZ 25 mg/kg was administered using an intragastric cannula and four mice (n = 4) were used for each of the assayed times. Blood samples were collected in heparinized plastic tubes at time 0 (pretreatment) and in the following post-treatment times: 0.08, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 10, 12, 15 and 24 h. Blood samples were centrifuged at 2000 × *g* and 4°C for 15 min and the recovered plasma was stored at -20°C until analysis by HPLC.

Analysis of the ABZ sulfoxide metabolite in plasma

The quantification of ABZ and its sulfoxide metabolite isolated from plasma was performed by an HPLC methodology described in a previous work [12,21]. The concentration versus time curve for the metabolite ABZSO in plasma for each animal group after the different treatments was fitted using PK Solution 2.0 (Summit research services, OH, USA). The peak concentration (C_{max}) and time to peak concentration were obtained from the plotted concentration–time curve of each analyte. The area under the concentration–time curve was calculated by the linear trapezoidal rule [22].

Statistics

In the PK study, a nonparametric Mann–Whitney test was used for the multiple statistical comparisons of the data obtained from the different animal groups. These statistical analyses were performed using the InfoStat software [23], considering a p-value < 0.05 as significant.

Results & discussion

Figure 1 shows the particle size and PDI variation during the HPH of S1 and S2 containing different ABZ:P188 ratios (1:0.5 and 1:1 respectively). When the homogenization pressure is raised to 1200 bar, a few amount of cycles was enough to achieve the submicron range, that is, at five cycles, the particle size was 935.69 ± 152.10 nm for

Table 1. Particle size and polydispersity index of nanosuspensions S1, S2 and its redispersed powdered forms PS1 and PS2. The spray-drying solid yield and moisture content of PS1 and PS2 are also shown.

Sample	Particle size (nm)	PDI	SY (%)	MC (%)
S1	420.23 ± 10.14	0.274 ± 0.007	–	–
PS1	550.16 ± 8.94	0.230 ± 0.020	<10	2.35
S2	372.43 ± 5.19	0.256 ± 0.005	–	–
PS2	415.69 ± 7.40	0.245 ± 0.006	72.32	3.18

MC: Moisture content; PDI: Polydispersity index; PS1: Powder from suspension 1; PS1: Powder from suspension 2; S1: Suspension 1; S2: Suspension 2; SY: Solid yield.

S1 and 820.43 ± 89.52 nm for S2 with a minimum at 30 cycles of 420.43 ± 10.14 nm and 372.43 ± 5.19, respectively. The PDI was also improved during the process with a value of 0.274 ± 0.007 (S1) and 0.256 ± 0.005 (S2) at 30 cycles. In both cases, the particle size diminution is bigger during the first 10–15 cycles while a decrease in nanonization rate is observed after 20 cycles, probably because of the exhaustion of weak points in the drug particle [2].

After HPH, the nanosuspensions were spray dried and the PY, MC and redispersion capacity were measured. The obtained powders from S1 and S2 (PS1 and PS2, respectively) presented low MC values (2.35 and 3.18%, respectively) and this was related to the conditions used in the SD experiments. A high atomization air pressure (819 l/h) and low feed rate (pump: 5%) allowed the generation of very fine droplets and consequently, a large specific surface when the suspension was sprayed in the drying chamber, thus permitting an efficient loss of water. On the other hand, a high redispersion capacity was observed since the particle size and PDI of PS1 and PS2 were similar to the correspondent original suspensions (see Table 1); this was attributed to the presence of P188 soluble links between the drug particles, whose dissolution allowed the formation of nano-sized ABZ dispersions upon contact with water. The concentration of P188 also influenced the recovery of solids in the collector as an augmented proportion of this polymer in the suspension produced a sharp increase in SY. This was related to the increased amount of solids in the sprayed droplet, which produced an augmented density of the particles, thus facilitating its separation in the cyclone.

With the aim of improving the SY of S1 and maintaining its redispersion capacity, 1.25 g of carrier materials L and CSD were added to the suspension at different ratios. As shown in Figure 2, when the amount of L was 100%, the sample presented enough redispersion performance with a particle size of 453.1 nm, but on the other hand, the SY was poorly increased (21.28%). Otherwise, samples containing lower L-CSD ratios produced greater SY, that is, 75–25: 47.96%; 60–40: 53.52% and 50–50: 67.52%. CSD is a highly porous material, which permits a rapid water removal, increasing the drying efficiency. Nevertheless, this improvement tendency in the SY was detrimental to the ability of redispersion since the powdered material particle sizes increased, that is, L-CSD 60–40: 641.03 nm and L-CSD 50–50: 919.20 nm. Based on the observed results, and considering the high PY and redispersion capacity of PS2, composition of which was ABZ and P188 at a ratio of 1:1, it was chosen for further investigation.

Since low aqueous solubility of ABZ may limit its absorption during GI transit, the study of its dissolution rate in the stomach is thought to be pivotal. Thus, the biopharmaceutic behavior of the nano-sized formulation was assayed simulating the acidic conditions of the stomach (HCl 0.1 M).

In the dissolution assay (Figure 3), PS2 presented high dissolution rate (Q_{1h}: 74.43%), while PM and the pure ABZ did not exceed 10% at 1 h (Q_{1h}: 7.77% and Q_{1h}: 3.7%, respectively). 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 [24].

Poloxamers are polyoxyethylene–polyoxypropylene block copolymer nonionic surfactants that have been widely used as wetting and solubilizing agents. The polyoxyethylene segment is hydrophilic whereas the polyoxypropylene segment is hydrophobic. The high percentage of P188 in the formulation (50% w/w) permits the formation of the previously described water-soluble links, 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 gelatine capsules within 10 min of the test. It is important to note that P188 has the disadvantage of forming *in situ* thermosensitive gels that might delay the drug release from the formulation, as reported by Dib *et al.* [25]. Nevertheless, according to this study, this behavior seemed not to affect drug dissolution from the gelatin capsules. Regarding the potential surfactant effect of P188 in the dissolution rate of ABZ it is negligible, which can

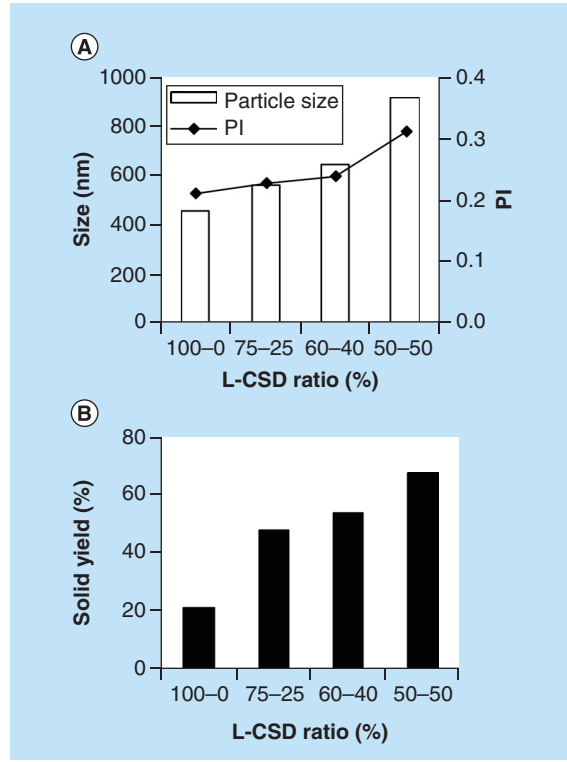

Figure 2. Re-dispersion particle size and spray-drying solid yield of S1 added with L and colloidal silicon dioxide (CSD) at different ratios.

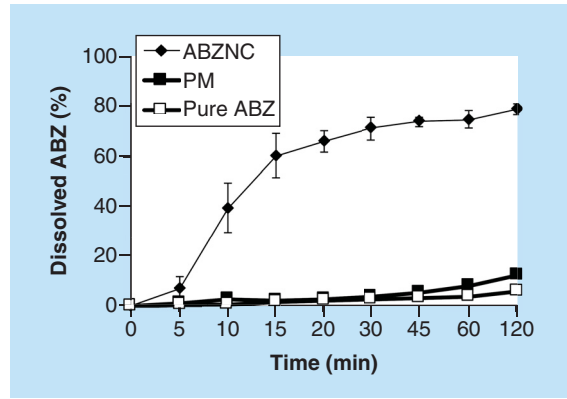

Figure 3. Dissolution profile of albendazole nanocrystals (PS2). Physical mixture and pure albendazole in 900 ml of HCl 0.1 N, apparatus 2, 75 rpm at 37°C.
ABZNC: ABZ nanocrystal.

be confirmed analyzing Figure 3 where pure ABZ and the PM (containing 50% of P188) presented very similar dissolution profiles.

On the other hand, amorphization 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 ABZNC [20].

However, the higher dissolution rate observed for the ABZNC does not necessarily guarantee an improvement in pharmacokinetic parameters, making further *in vivo* studies necessary.

Pharmacokinetics

When administered orally, ABZ is extensively metabolized to an active sulfoxide metabolite (ABZSO) in the liver, and further oxidation leads to the second inactive metabolite, ABZ sulfone [26]. To achieve a suitable plasma concentration of the ABZSO, ABZ (a weak base) must dissolve in the acid environment of the stomach to finally permeate the duodenal membrane by a passive diffusion process which is independent of the administered dose [6]. NC of basic drugs are more easily affected by pH variation in the GI tract. For weak bases, a nano-sized drug formulation will dissolve fast and more efficiently in the low stomach pH environment, but in the

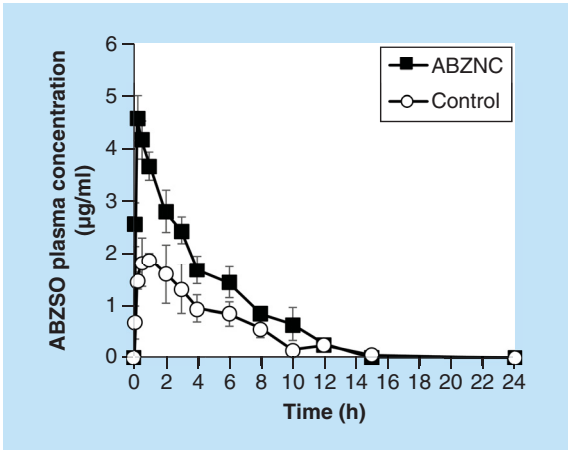

Figure 4. Pharmacokinetic profile of ABZNC and PM in mice after asingle intragastric administration of albendazole (ABZ; 25 mg/kg).
ABZNC: ABZ nanocrystal; ABZSO: Albendazole sulfoxide.

Table 2. Pharmacokinetic parameters of the sulfoxide metabolite (albendazole extensively metabolized to active sulfoxide metabolite) after a single oral administration of albendazole nanocrystals and control formulation at 25 mg/kg.

	T_{max} (h)	C_{max} (µg/ml)	$AUC_{0\rightarrow\infty}$ (µg.h/ml)
ABZNC	0.31 ± 0.13†	4.76 ± 0.17†	19.70 ± 0.45†
Control (PM)	1.25 ± 0.87	2.14 ± 0.12	10.71 ± 0.90

Data are expressed as mean ± SD (n = 6).
† $p < 0.05$ vs control.
ABZNC: Albendazole nanocrystal; ABZSO: Albendazole sulfoxide; $AUC_{0\rightarrow\infty}$: Area under the plasma concentration–time curve up to time infinity; C_{max}: Peak plasma concentration; T_{max}: Time to reach peak plasma concentration.

transit to duodenum the rise in pH may illicit uncontrolled precipitation of drug substance [27]. Nonetheless, this phenomenon seemed not to affect our formulation since its pharmacokinetic performance was truly increased. Although the GI transit in mice is rapid, we hypothesized that the gastric emptying is gradual, thus permitting the absorption of the active before achieving its saturation concentration in the duodene and its consequent precipitation.

It has been reported that the pharmacokinetic profiles of BZD anthelmintics may differ depending on the animal species. The difference in the anatomic features could influence the passage of digesta through the GI tract and hence, affect the pharmacokinetic performance of such molecules [12].

Figure 4 shows the pharmacokinetic profiles of ABZSO after a single oral administration of ABZNC and the control formulation at 25 mg/kg. ABZ was not detected in the plasma, whereas ABZSO was detected in both treatments up to 15 h. ABZNC produced a rapid increase in the sulfoxide plasma concentration, achieving a maximum at 0.31 ± 0.13 h, while the control presented the C_{max} at 1.25 ± 0.87 h. The area under the plasma concentration–time curve up to time infinity value for ABZNC (19.70 ± 0.45 µg.h/ml) was almost twice that obtained for the control group (10.71 ± 0.90 µg.h/ml). The C_{max} value observed for ABZNC was 1.2-fold higher ($p < 0.05$) than the control (4.76 ± 0.17 µg/ml and 2.14 ± 0.12 µg/ml, respectively) (see Table 2). This augmented plasma exposure of ABZSO could lead to an improvement in the treatment of echinococcosis (hydatid cyst) in agreement with the results previously obtained by our group when ABZ prepared as solid dispersions was administered in a murine model [7]. Moreover, the potential of ABZ to induce an antitumor effect by inducing oxidative cleavage on DNA has been recently discussed [28]; in this sense, the results here exposed could have a tremendous impact on public health.

As shown in Figure 5, an optimized NC formulation produced by the combination of HPH and SD must have a suitable balance between the feasibility of the drying process (i.e., SY), the redispersion capacity of the obtained powdered material (particle size and PDI) and the final drug percentage formula (drug loading). Here, we were able to obtain ABZNC through a potentially scalable and inexpensive process. This material was also capable of magnify ABZ absorption in the GI tract.

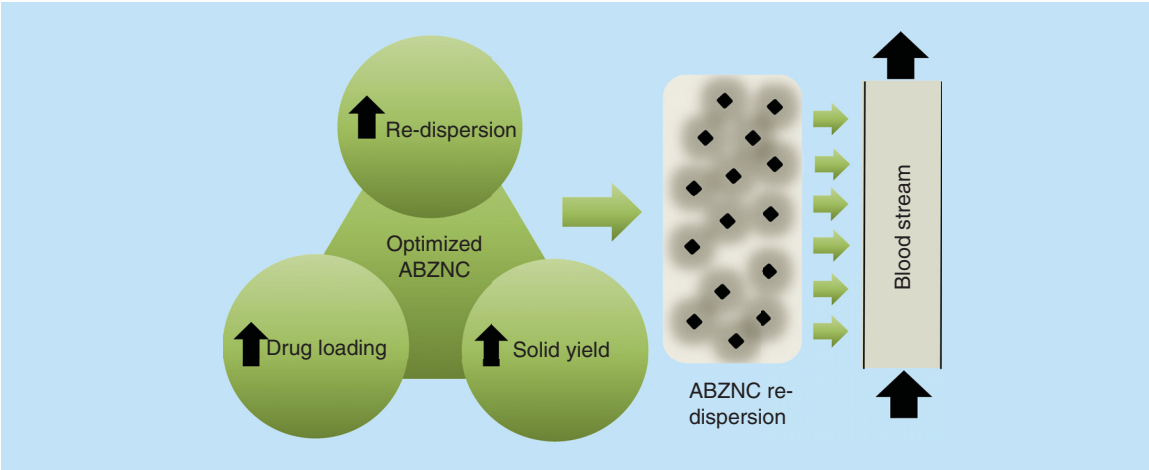

Figure 5. An optimized formulation of ABZNC must balance a high re-dispersion and solid yield maintaining a high drug-loading capacity. The enlarged specific surface enables an increased in the dissolution rate, saturation concentration and mucoadhesivity, leading to enhanced albendazole plasma exposure.
ABZNC: ABZ nanocrystal.

Conclusion

ABZNCs were obtained by means of a combined process of premilling (three cycles at 500 bar) and HPH (30 cycles at 1200 bar). Then, using an optimized SD process, this dispersion was transformed into a highly redispersible powder able to be incorporated into solid pharmaceutical dosage forms (tablet, capsule). ABZNC were capable of increase the oral absorption of the drug in a murine model.

Future perspective

Based on the promising outcomes of the pharmacokinetic assay, it would be propitious to move forward with further *in vivo* efficacy studies aimed at evaluation of the potential of ABZNC in the treatment of hydatid cyst in humans or ancylostomiasis, a zoonotic parasitic disease that is highly prevalent in dogs. On the other hand, regarding the potential of ABZ as an antitumor agent, an increase in the bioavailability could be very beneficial. Moreover, the flexibility of the processes involved here could permit the formulation of other antiparasitic BZD drugs with poor aqueous solubility such as fenbendazole or flubendazole.

Executive summary

- Albendazole (ABZ) is a broad-spectrum antiparasitic agent used in human and veterinary medicine with a poor aqueous solubility.
  - ABZ acts by preventing tubulin aggregation of eukaryotic cells.
  - The low solubility of ABZ leads to poor oral absorption when administered orally.
  - The lack of oral absorption has been linked to therapeutic failures following oral administration.
- Nanocrystals (NC) are defined as nanoparticles (typically 200–500 nm) composed of practically 100% drug.
  - NC are generally produced as aqueous dispersions (nanosuspensions), which usually need further solvent removal in order to obtain redispersible powders.
  - NC display 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.
- When ABZ is formulated as NC, a sharp increase in the specific surface is produced, which leads to increased dissolution rate, saturation concentration and mucoadhesion.
- An improved PK performance of the nano-sized formulation was observed in mice; this could potentially enhance the therapeutic response of certain parasitic diseases (i.e., hydatid cyst, ancylostomiasis) to ABZ.

Acknowledgements

The technical assistance of C Farías from CIVETAN in the processing of HPLC samples is also acknowledged.

Financial & competing interests disclosure

The authors wish to acknowledge the assistance, the financial support and facilities from the National Scientific and Technical Research Council (CONICET), National University of Central Buenos Aires and National University of Córdoba. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The protocols for animal procedures and management were approved by the Ethics Committee according to the Animal Welfare Policy of the Faculty of Veterinary Medicine, National University of Central Buenos Aires, Tandil, Argentina (www.vet.unicen.edu.ar).

References

Papers of special note have been highlighted as: • of interest

1 Zhou Y, Du J, Wang L, Wang Y. State of the art of nanocrystals technology for delivery of poorly soluble drugs. *J. Nanoparticle Res.* 18(9), 1–22 (2016).

2 Keck CM, Müller RH. Drug nanocrystals of poorly soluble drugs produced by high pressure homogenisation. *Eur. J. Pharm. Biopharm.* 62(1), 3–16 (2006).

• **It describes in depth the cavitation phenomenon which is responsible for the particle nanonization in the high pressure homogenization process.**

3 Müller RH, Gohla S, Keck CM. State of the art of nanocrystals – special features, production, nanotoxicology aspects and intracellular delivery. *Eur. J. Pharm. Biopharm.* 78(1), 1–9 (2011).

4 Shegokar R, Müller RH. Nanocrystals: industrially feasible multifunctional formulation technology for poorly soluble actives. *Int. J. Pharm.* 399(2), 129–139 (2010).

5 Mohana K, Srivalli R, Mishra B. Drug nanocrystals: a way toward scale-up. *Saudi Pharm. J.* 24(4), 386–404 (2016).

6 Lacey E. Mode of action of benzimidazoles. *Parasitol. Today* 6(4), 112–115 (1990).

7 Pensel PE, Castro S, Allemandi D, Bruni S Sanchez, Palma SD, Elissondo MC. Enhanced chemoprophylactic and clinical efficacy of albendazole formulated as solid dispersions in experimental cystic echinococcosis. *Vet. Parasitol.* 203(1), 80–86 (2014).

8 Moreno-Esparza R, Jung H, Medina L, García L, Fuentes I. Absorption studies of albendazole and some physicochemical properties of the drug and its metabolite albendazole sulphoxide. *J. Pharm. Pharmacol.* 50(1), 43–48 (1998).

9 Abulaihaiti M, Wu X, Qiao L *et al.* Efficacy of albendazole–chitosan microsphere-based treatment for alveolar echinococcosis in mice. *PLoNegl S. Trop. Dis.* 9(9), e0003950 (2015).

10 Mingjie W, Shuhua X, Junjie C *et al.* Albendazole–soybean oil emulsion for the treatment of human cystic echinococcosis: evaluation of bioavailability and bioequivalence. *Acta Trop.* 83(2), 177–181 (2002).

11 Priotti J, Codina AV, Leonardi D *et al.* Albendazole microcrystal formulations based on chitosan and cellulose derivatives: physicochemical characterisation and *in vitro* parasiticidal activity in *Trichinella spiralis* adult worms. *APharmSciTech APS* 18(4), 947–956 (2017).

12 Castro SG, Sanchez Bruni SF, Urbizu LP *et al.* Enhanced dissolution and systemic availability of albendazole formulated as solid dispersions. *Pharm. Dev. Technol.* 18(2), 1–9 (2012).

13 Wen H, New RR, Muhmut M *et al.* Pharmacology and efficacy of liposome-entrapped albendazole in experimental secondary alveolar echinococcosis and effect of co-administration with cimetidine. *Parasitology* 113(2), 111–121 (1996).

14 Palomares-Alonso F, Rivas C, Bernad-Bernad MJ *et al.* Two novel ternary albendazole – cyclodextrin – polymer systems: dissolution. bioavailability and efficacy against *Taenia crassiceps* cysts. *Acta Trop.* 113, 56–60 (2010).

15 Vogt M, Kunath K, Dressman JB. Dissolution improvement of four poorly water soluble drugs by co-grinding with commonly used excipients. *Eur. J. Pharm. Biopharm.* 68, 330–337 (2008).

16 Naseri M, Akbarzadeh A, Spotin A. Scolicidal and apoptotic activities of albendazole sulphoxide and albendazole sulphoxide-loaded PLGA-PEG as a novel nanopolymeric particle against *Echinococcus granulosus* protoscoleces. *Parasitol. Res.* 115(12), 4595–4603 (2016).

17 Kang BS, Choi JS, Lee SE *et al.* Enhancing the *in vitro* anticancer activity of albendazole incorporated into chitosan-coated PLGA nanoparticles. *Carbohydr Polym.* 159, 39–47 (2016).

18 Kudtarkar SKA, Shinde U, Bharkad GP. Solid lipid nanoparticles of albendazole for treatment of *Toxocara canis* infection: *in vivo* efficacy studies. *Nanosci. Nanotechnol. Asia* 7(2) 80–91 (2017).

19 Jelowdar A, Rafiei A, Abbaspour MR, Rahdar M. Efficacy of combined albendazol and praziquntel and their loaded solid lipid nanoparticles components in chemoprophylaxis of experimental hydatidosis. *Asian Pac. J. Trop. Biomed.* 7(6), 549–554 (2017).

20 Paredes AJ, Llabot JM, Bruni S Sánchez, Allemandi D, Palma SD. Self-dispersible nanocrystals of albendazole produced by high pressure homogenization and spray-drying. *Drug Dev. Ind. Pharm.* 42(10), 1564–1570 (2016).

21 Sánchez SF, Alvarez LI, Lanusse CE. Nutritional condition affects the disposition kinetics of albendazole in cattle. *Xenobiotica* 26(3), 307–319 (1996).

22 Gibaldi M, Perrier D. *Pharmacokinetics (2nd Edition).* Marcel Dekker, Inc., NY, USA (1982).

23 Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW. InfoStat. Universidad Nacional de Córdoba. Córdoba, Argentina. (2016).

24 Mauludin R, Müller RH, Keck CM. Development of an oral rutin nanocrystal formulation. *Int. J. Pharm.* 370, 202–209 (2009).

25 Dib A, Palma S, Suárez G *et al.* Albendazole sulphoxide kinetic disposition after treatment with different formulations in dogs. *J. Vet. Pharmacol. Ther.* 34(2), 136–141 (2011).

26 Sanchez Bruni SF, Jones DG, McKellar QA. Pharmacological approaches towards rationalizing the use of endoparasitic drugs in small animals. *J. Vet. Pharmacol. Therap.* 29, 443–457 (2006).

27 Gao L, Liu G, Ma J, Wang X, Zhou L, Li X. Drug nanocrystals: *in vivo* performances. *J. Control. Rel.* 160(3), 418–430 (2012).

• **Complete review involving the versatility of nanocrystals and their ability to increase bioavailability and efficacy in *in vivo* models.**

28 LSCastro EPW, Kviecinski MR, Ourique F *et al.* Albendazole as a promising molecule for tumour control. *Redox Biol.* 10, 90–99 (2016).