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Albendazole-lipid nanocapsules: Optimization, characterization and chemoprophylactic efficacy in mice infected with *Echinococcus granulosus*

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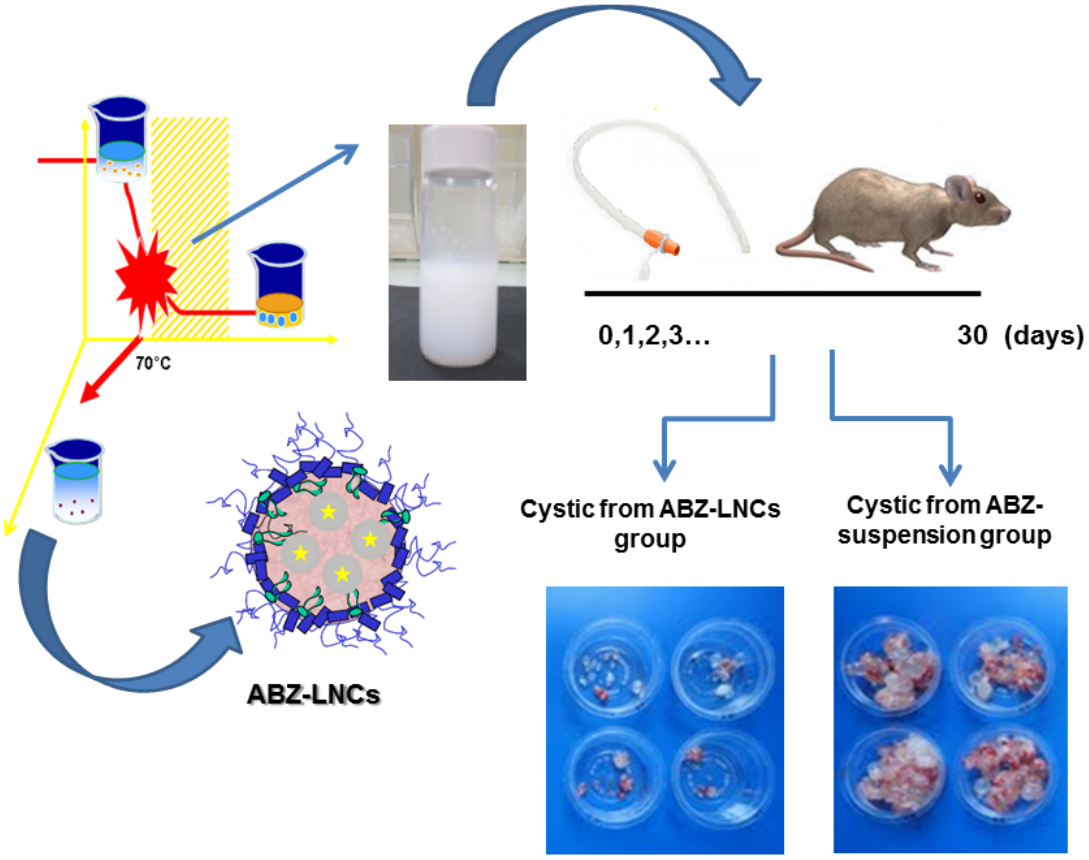
70°C
ABZ-LNCs
0,1,2,3…
30 (days)
Cystic from ABZ-LNCs group
Cystic from ABZ-suspension group

Albendazole-lipid nanocapsules: optimization, characterization and chemoprophylactic efficacy in mice infected with *Echinococcus granulosus*

ABSTRACT

Cystic echinococcosis (CE), which is caused ~~by~~ during the metacestode larval stage of *Echinococcus granulosus*, is a life-threatening disease and is very difficult to treat. At present, the FDA-approved antihelmintic drugs are mebendazole (MBZ), albendazole (ABZ) and its principal metabolite ABZ sulfoxide (ABZSO), but as these have ~~with~~ a therapeutic efficacy over 50%, underlining the need for new drug delivery systems. The aim of this work was the optimization and characterization of ~~to optimised and fully characterizes the~~ previously developed ABZ lipid nanocapsules (ABZ-LNCs) ~~previously developed~~ and evaluate their efficacy in mice infected with *E. granulosus* ~~the murine model of CE~~. LNCs were prepared by the phase inversion technique and characterized in terms of size, surface charge, drug loading, and *in vitro* stability followed by an in vivo proof-of-concept ~~performed in~~ using a ~~CE murine model~~ murine model infected with E. granulosus. Stable particle~~s~~ dispersions with a narrow size distribution and high efficiency of encapsulation (≥90%) were obtained. ABZ-LNCs showed a greater chemoprophylactic efficacy than ABZ suspension administered by the oral route ~~since~~ as 4 out of the 10 ABZ-LNCs treated mice did not develop any cysts, whereas the infection progressed in all mice from the ABZ suspension group. Regarding the ultrastructural studies of cysts, mice treated with ABZ-LNCs or ABZ suspension revealed changes in the germinal layer. However, the extent of the damage appeared to be greater after ABZ-LNC~~s~~ administration compared to the suspension treatment. These results suggest~~ed~~ that ABZ-LNCs ~~are~~ could be a promising novel candidate for ABZ delivery to treat CE.

Keywords

Albendazole; Chemoprophylactic efficacy; Cystic echinococcosis; Drug delivery; Lipid nanocapsules.

1. INTRODUCTION

Cystic echinococcosis (CE), a zoonosis caused by the larval stage of *Echinococcus granulosus*, is characterized by the long-term growth of cysts in humans and mammalian intermediate hosts (McManus et al., 2012). This is a chronic and complex parasitic infection ~~is a chronic, complex,~~ and still neglected disease (Brunetti and Junghanss, 2009). Currently, four treatment approaches are used ~~in use:~~ surgery, PAIR (puncture, aspiration, injection of protoscolicidal agent and reaspiration), chemotherapy with benzimidazoles (BZ), and watching and waiting for the appearance of inactive, clinically silent cysts (Stojkovic et al. 2009). Albendazole (ABZ) and mebendazole (MBZ) are the BZ commonly indicated for inoperable patients with multiple cysts in two or more organs and, also for the prevention of secondary echinococcosis after surgery (Pawłowski et al., 2001). According to WHO recommendations, ABZ should be administered in daily doses of 10-15 mg/kg of body weight taken in two divided doses post-prandially for 3-6 months (WHO Informal Working Group on Echinococcosis, 2001). Nevertheless, approximately only one-third of patient's experience~~s~~ complete remission or cure, with ~~and~~ 30-50% of treated patients developing some evidence of a therapeutic response (Moro and Schantz, 2009). Despite this questionable efficacy, ABZ remains the best treatment option for inoperable human cases, and is the drug of choice for perioperative prophylaxis due to the lack of alternative drugs against hydatid cysts.

Regarding the oral route, the low aqueous solubility coupled ~~to~~ with the slow dissolution rate of ABZ ~~generally~~ leads to a poor and erratic absorption from the gastro-intestinal tract. (Alanazi et al. 2007; Martinez-Marcos et al. 2016; Castro et al. 2010). Thus, several options are currently being explored in order to overcome these drawbacks ~~improve ABZ solubility~~. One ~~A~~ viable strategy is the choice of a dissolution medium that allow the ionization of this molecule ~~in acidic medium since the drug is basic in nature~~ but this solubility enhancement is not enough for preparing formulations containing the required ~~high~~ ABZ concentration (Garcia et al. 2003). Another alternative is the use of

surfactants, such as polysorbate and bile salts (del Estal et al. 1994; Torrado et al. 1996). However, the detergents properties of these formulations are associated with irritation of the digestive mucosa. Similarly, the complexation with cyclodextrins (Pradines et al., 2014) or co-grinding ABZ with various excipients (Pluronic 188®, lactose monohydrate, corn starch, polyvinylpyrrolidone, hydroxypropylmethyl cellulose and sodium lauryl sulphate) using jet-milling and solid dispersion techniques (Castro et al., 2012; Vogt et al., 2008) were also tested without any clear benefits being found.

In relation With respect to CE, it is important to highlight that the success of the post-surgical chemoprophylactic treatment is based upon the capacity of ABZ to operate on inhibit the protoscoleces in order to avoid their establishment and the development to of cysts. Therefore, the development of novel formulations that facilitate the controlled able to control the drug release of the drug to the target site is still now a an ongoing challenge. Nanotechnology-based delivery systems have emerged as promising alternatives to for improving the therapeutic efficiency of ABZ based on its by selective targeting and a tunable delivery rate, although their *in vivo* effectiveness has was not been extensively studied (Kang et al., 2015; Liu et al., 2013; Mukherjee and Plakogiannis, 2010; Press, 2010). Among these, lipid nanoparticles were developed according to using a phase inversion process that leads follows the formation of an oil/water microemulsion containing an oily fatty phase, surrounded by a rigid tensioactive shell (Heurtault et al. 2002). These particles are were synthesized without the use of an organic solvent with a narrow size distribution which could be adjusted through precise modifications in pharmaceutically acceptable excipient proportions (Hirsjärvi et al., 2013; Huynh et al., 2009). Previously, it has been was shown that orally-administered LNCs can permeate through the mucus, increase drug absorption by the epithelial tissue, and finally, increase drug bioavailability (Roger et al., 2009a, 2009b, 2017). Concerning the CE, ABZ loaded LNCs were reported to improved the bioavaibility of ABZ in the plasma and cysts in of infected mice, wich and this was correlated with an increased clinical efficacy of the drug

(Pensel et al., 2015). Taking into account these above findings ~~considerations~~, this study was conducted in order to optimize~~d~~ ABZ loaded LNCs and to characterize~~d~~ them in terms of size, surface potential, encapsulation efficiency, and *in vitro* drug stability. The chemoprophylactic efficacy of this formulation was then evaluated in mice infected with *E. granulosus*.

2. MATERIALS AND METHODS

2.1. Materials

ABZ powder was purchased ~~to~~ from Parafarm (Buenos Aires, Argentina) and Captex 8000® (tricaprylin) was supplied by Abitec Corp. (Colombus, Ohio, USA). The lipophilic Labrafac® WL 1349 (caprylic-capric acid triglycerides; European Pharmacopeia, IVth, 2002) and Transcutol HP® (diethylene glycol monoethyl ether) were kindly provided by Gattefossé S.A.S (Saint-Priest, France). Lipoid® S75-3 (soybean lecithin ~~at~~ with 70% of phosphatidylcholine and 10% of phosphatidylethanolamine) and Koliphor® HS-15 (mixture of free polyethylene glycol 660 and polyethylene glycol 660 hydroxystearate) were gifts from Lipoid Gmbh (Ludwigshafen, Germany) and BASF (Ludwigshafen, Germany), respectively. Due to the complex composition of each product, the brand names will be used from here on ~~in the following text~~. NaCl was purchased from the Cicarelli lab (Buenos Aires, Argentina), and oleic acid (OA), monobasic ammonium phosphate, polisorbate 80 (Tween 80®) and Carboxymethilcellulose (CMC)~~,~~ were purchased from Sintorgan (Buenos Aires, Argentina). Porcine pepsin and pancreatin (reagent grade) enzymes ~~were American Chemical Society (ACS) and~~ were bought from Sigma (St. Louis, MO, USA). Purified water was obtained from MilliQ System (Biopore, Buenos Aires, Argentina). All other chemical reagents were of HPLC-grade and acquired from Sintorgan, Argentina.

2.2. LNC~~s~~ formulations

The LNCs were prepared according to a ~~the~~ process described by Heurtault et al., 2002 ~~including~~ with a few modifications. First~~ly~~, ABZ was solubilised in OA (25 mg/g). Then, 0.8 g of the

oily matrix (Labrafac®, Captex®8000 or a mix of them) was added, and the mixture was heated at 80 ºC for 5 min. After cooling, Lipoid® S75-3 (0.075g), Kolliphor ®HS-15 (0.846g), NaCl (0.089g) and water (2.96 g) were added and homogenized under magnetic stirring. Three cycles of progressive heating and cooling between 60-85°C were carried out ~~and~~ which was followed by an irreversible shock induced by dilution ~~with~~ through the addition of deionised water (12.5 g) ~~added to the mixture~~ at 75 °C. Afterwards, a slow magnetic stirring was applied to the suspension of LNCs for 5 min at room temperature. The final concentration of ABZ was 0.29 mg/g.

2.3. Particle size and zeta potential (ZP) measurements

The particle size, based on ~~a~~ volume distribution values, (D90), the polydispersity index (PI)~~,~~ and zeta (ζ) potential were determined by dynamic light scattering (DLS) on a DelsaNano-C instrument (Beckman Coulter, Osaka, Japan) fitted with a 488-nm laser beam at a fixed angle (90°) at 25°C ~~with~~ using DelsaNano 2.20 (Beckman Coulter, Osaka, Japan) instrument software. All measurements were performed in triplicate and nanocapsules were diluted 1:20 (v/v) ~~in~~ with deionised water.

2.4. Scanning electron microscopy (SEM)

The morphological structures of LNCs were investigated using ~~a~~ the scanning electron microscope LEO Model EVO 40XVP (Göttingen, Germany). The nanoparticle suspensions were diluted (1:400) in distilled water and fixed on a brass stub using a double-sided aluminium tape. To improve the conductivity, samples were gold-coated under vacuum by employing a sputter coater PELCO Model 3.

2.5. LNC~~s~~ drug payload and encapsulation efficiency

After formulation, LNCs were filtered using a Minisart® 0.2 μm filter (Vivascience AG, Hanovre, Germany) in order to eliminate ~~not~~ unencapsulated ABZ crystals~~.~~ and samples were prepared by dissolving an exact quantity of these filtered LNCs in methanol. The ABZ concentration

was measured by liquid chromatography (HPLC) in triplicate experiments. A Waters HPLC 1525 pump with a Waters 717 plus autosampler was used, and 20 µl aliquot of the filtrate was injected into a reversed phase C18 column (250×4.6mm i.d., 5µm particle size, Luna, Phenomenex®). The oven temperature was maintained at 40 °C using a Waters column heater 1500 series. The assay ~~analysis~~ was performed with 0.01M monobasic ammonium phosphate and methanol (40:60) as mobile phase, at a flow rate of 1.3 ml/min. Eluting fractions were revealed with a PDA UV detector 2296 (lambda 295 nm). The mean ± SD drug content was calculated (mg of ABZ/g LNCs dispersion)~~.~~ and the efficiency of encapsulation (EE%) was determined by dividing the experimental by the theoretical drug loading.

2.6. Storage stability studies

The stability of ABZ-loaded LNC~~s~~ dispersion was evaluated after storage at 4-8 ºC for 2 months~~.~~ and the particle size distribution and drug loading were determined as ~~previously~~ described above.

2.7. Stability of the nanocapsules in simulated gastrointestinal fluids

As these nanostructures were developed as carriers intended for oral administration of ABZ, ~~thus~~ we evaluated their stability in the gastrointestinal fluids. An aliquot (50 µl) of ABZ-LNCs (0.29 mg/g) ~~were~~ was placed in a glass tube in triplicate and incubated at 37 °C under moderate stirring (100 rpm), either in 2.95 ml of simulated gastric medium (USP XXVII, pH=1.2, pepsin 0.32% w/v) ~~and~~ or in simulated intestinal medium (USP XXVII, pH=6.8, pancreatin 1% w/v). Samples from each tube were collected at a predetermined incubation time and centrifuged for 5 min at 5000 g in order to precipitate the enzymes. Then, the mean particle size of the remaining non-aggregated nanocapsules was determined by DLS. In order to check the ABZ loading, the samples were filtered after incubation using a Minisart® 0.2 µm filter (Sartorius, Goettingen, Germany) to remove free

153 precipitated ABZ, and the drug payload was determined by HPLC in triplicate as ~~it was~~ described in
154 section 2.5.

155 **2.8. Animal studies**

156 Animal procedures and management protocols were approved by the Institutional Animal
157 Care and Use Committee (RD RD 148/15) of the Faculty of Exact and Natural Sciences, National
158 University of Mar del Plata (Mar del Plata, Argentina), and carried out in accordance with the 2011
159 revised form of The Guide for the Care and Use of Laboratory Animals published by the U.S.
160 National Institutes of Health. Any unnecessary animal suffering was avoided throughout the study.
161 Female CF-1 mice (n=40; body weight 25±5g) were infected by intraperitoneal inoculation with
162 1500 *E. granulosus* protoscoleces/animal, suspended in 0.5 ml of medium 199 (Gibco, Thermo
163 Fisher Scientific, Argentina). The animals were housed in a temperature-controlled (22 ± 1 °C),
164 light-cycled (12-hour light/dark cycle) room. Food and water were given *ad libitum*.

165

166 **2.8.1. Protoscoleces collection**

167 Protoscoleces of *E. granulosus* were collected aseptically from liver and lung hydatid cysts of
168 infected cattle slaughtered ~~in~~ at an abattoir located in the southeast of Buenos Aires, Argentina.
169 Viability was assessed by the methylene blue exclusion test (Elissondo et al., 2007).

170

171 **2.8.2. ABZ formulations**

172 Suspensions were prepared in 100 ml of purified water with the required amount of
173 suspending agent (CMC) and kept overnight for proper hydration. This solution was used as vehicle
174 in the preparation of the suspensions. An accurately weighed quantity of ABZ was distributed in the
175 vehicle, and Tween 80® was added and mixed gently for 30 min. The ABZ suspension (0.29 mg/g)
176 was vigorously shaken before its intragastric administration to mice. The ABZ-LNCs were prepared
177 as described above at an equal concentration.

2.8.3. Chemoprophylactic efficacy study

Twenty-four hours after ~~the~~ infection, CF-1 mice were allocated ~~in~~to four experimental groups (10 animals/group) and treated as follows: a) Control group, animals receiving distilled water as placebo; b) Control LNC~~s~~ group, animals receiving blank LNCs; c) ABZ suspension treated group; d) ABZ-LNC~~s~~ treated group. The treatment was performed daily for 30 days by intragastric administration (0.55 ml/animal) at a dose rate of 5 mg/kg. Six months after infection, mice were euthanized, and necropsy was carried out immediately ~~thereafter~~.

2.8.4. Determination of parasite weight and efficacy rate of treatments

At necropsy, the peritoneal cavity was opened~~,~~ and the cysts were carefully removed. ~~With~~ The weight of the cysts collected from each individual animal ~~was~~ being recorded using an analytical scale. The efficacy of treatments, based on the weight of the cysts from infected mice, was calculated using the following formula: (mean cyst~~s~~ weight of control group - mean cyst~~s~~ weight of treated group)/ (mean cyst~~s~~ weight of control group) x 100.

2.8.5. Morphological study

Samples of cysts recovered from each mouse were processed by SEM as described elsewhere (Elissondo et al., 2007).

Statistical analysis

Cysts weight~~s~~ (mean ± SD) were compared by means of the Kruskal–Wallis (non parametric ANOVA) test followed by Dunn's multiple comparison test~~.~~ with a value of $P<0.05$ ~~was~~ being considered to be statistically significant. The statistical analysis was performed using the Instat 3.0 software program (GraphPad Software, San Diego, CA).

3. RESULTS

3.1. Development and formulation ~~set up~~

The first goal in the formulation process was to find ~~out~~ the most convenient core material able to solubilise a sufficient amount of ABZ. As this drug is sparingly soluble in water and in the most common organic solvents (Torrado et al., 1996). ~~Preliminarily,~~ The solubility of ABZ in HCl 0.1 M and further encapsulation into the oily inner phase were evaluated since this strategy was reported as being successful for another hydrophobic drug (Roger et al., 2011). However, in our study ~~this case~~, drug loading and EE% were low and were considered ~~not~~ unacceptable for further applications. ~~Alternatively, ABZ could be solubilised in~~ Thus, co-surfactants and oils were tested but ~~added to the preparation. Results of this study showed that among the oils and co-surfactants tested~~ only OA and Transcutol HP® were able to solubilise ABZ.

It is important to highlight that the thermodynamic stability of LNCs is strongly dependent on the nature of the lipid material utilised as the core. Therefore, ~~the next step was to~~ we evaluated the combination of the lipids ~~such as~~ Labrafac® or Captex® 8000 and the co-surfactants OA and Transcutol HP®. Regarding the latter co-surfactant, ~~last one,~~ as only a small quantity of this excipient (≤0.10 g/g lipid) combined with Labrafac® allowed the production of stable LNCs formulation consequently, this excipient was discarded. In the case of OA, the maximal amount which leads to LNC~~s~~ suspension with acceptable physical properties was estimated in 0.25 g/g lipid. Next, in order to evaluate the efficiency of this material as an ABZ solubiliser, three batches of LNCs (A, B, and C; n=3) were manufactured using Labrafac®, Captex® 8000 and a mixture Labrafac®/Captex® 8000 (50/50) respectively as the lipid core and then ~~mixing~~ each one was mixed with OA. Their physical properties were determined and are listed in Table 1. A blank LNC~~s~~ suspension using Captex®8000 and OA as the lipid core was included as a reference.

[Table 1]

~~We obtained~~ A monodisperse suspension (PI < 0.2) was obtained with a mean particle size ranging from 47-56 nm and with a negative zeta potential in all batches. Nevertheless, ~~only in Captex 8000® formulations~~ the EE% of ABZ was higher than 90% only in Captex 8000® formulations (batch B, table 1). Moreover, as these physical-chemical features were stable for at least for 1 month; ~~consequently,~~ the composition of batch B was selected for further studies.

3.2. Morphology of ABZ-LNCs

SEM was used to characterise the morphology of the optimised nanoparticles. ~~According to~~ In Fig. 1.a and 1.b, the observed diameter slightly differed from the values of the DLS determinations since the LNCs flattened during the drying stage of the ~~in~~ sample preparation, ~~This observation~~ whichseems to ~~be~~ frequently occur in the study of LNCs by means of this technique (Lamprecht et al., 2004). The SEM images in Fig. 1.b also ~~showed~~ reveal the homogeneity of the LNCs, although a few large particles ~~of higher size~~ can be observed.

[Figures 1.a and 1.b]

3.3. Storage stability

Particle stability ~~of particles~~ was assessed for 2 months by storing three batches of the obtained suspensions at 4-8 ºC. As shown in Table 2, the ABZ loaded in LNCs were physically stable for at least ~~for~~ 2 months ~~and~~ with no ~~significant~~ changes in mean particle size ~~and~~ or ZP ~~were~~ being observed. For all formulations, ~~the~~ PI was <0.2, which demonstrates the monodispersity of the preparations ~~and the~~ with the EE% of ABZ ~~was~~ being higher than 90%. The drug payload (~0.29 mg/g) remained constant and there were no significant differences between the evaluated batches (P<0.05).

[Table 2]

3.4. In vitro stability in simulated gastrointestinal fluids

~~In view of an oral administration of this formulation,~~ Considering that this formulation has to be administered orally, *in vitro* stability studies ~~in~~ on different simulated gastrointestinal fluids were first performed. In the gastric medium, which was characterized by a pH of 1.2 and the presence of a digestive protease (pepsin), a release of about 7% of the initial amount of encapsulated ABZ was measured after 3 h (Fig. 2). Then, the stability of ABZ-LNCs was assayed in simulated intestinal fluid media. As ~~it was seen~~ occurred before, ABZ remained encapsulated in the LNCs after 3 h of incubation (with less than 10% ~~was~~ being released). Also, the size and PI of the nanocarriers were monitored after incubation in these media (Fig. 3.a and 3.b). The slight~~ly~~ increase in size observed for the nanocapsules in the first medium was attributed to the presence of pepsin, since the system maintained its particle size in the absence of enzymes (data no shown). Regarding the incubation in simulated intestinal fluids, it is important to point out that the inclusion of pancreatin, as recommended by different pharmacopoeias, may have an unpredictable effect ~~over~~ on the lipid matrix of LNCs ~~since~~ as it is constituted by several enzymes such as amylase, lipase and protease which present diverse functions and catalytic sites (Prego et al., 2006). Nevertheless, because no further degradation was observed, ~~and~~ it seems that the chemical composition of these nanocapsules contributed to the stability of the systems in ~~these~~ this medium. We hypothesize that the shell composed of the association of free PEG and HS-PEG (Kolliphor® HS-15) in ~~their~~ its outer structure attached to the oily core improved the stability of the nanosuspension, which otherwise aggregated massively upon dilution in the incubation medium.

[Figure 2, 3.a and 3.b]

3.5. Chemoprophylactic efficacy study

All the infected mice (10/10) from the control groups and ABZ-treated group developed hydatidic cysts in the abdominal cavity, whereas ~~in 4 out of the 10 ABZ-LNCs treated mice~~ the infection did not progress in 4 out of the 10 ABZ-LNCs treated mice. A deleterious drug effect on *E. granulosus* protoscoleces at the time of infection may help to explain the lack of cyst development

observed in some animals of the ABZ-LNCs treated groups. The differences in cyst weight among experimental groups~~,~~ showing the intragroup variability~~,~~ are presented in Fig. 4. There ~~was~~ were no statistically significant differences (P>0.05) between the mean cyst~~s~~ weights of the control groups (distilled water =4.38±3.39 g; blank LNCs = 4.22±2.51 g). On the other hand, significant differences (P<0.005) were observed in the weight of the cysts recovered from untreated mice compared to that obtained from ABZ suspension (1.27±0.60 g) and ABZ-LNC~~s~~ (0.25±0.24 g) treatments. Interestingly, mice receiving ABZ-LNCs exhibited a higher reduction (P<0.05) in the weight of the cysts compared to ABZ suspension treated mice.

[Figure 4]

Fig. 5 shows the ultrastructural appearance of the germinal layer after an SEM analysis of cysts recovered from infected mice. All cysts in the samples removed from control mice appeared turgid, showing no observable collapse of the germinal layer and no change in ultrastructure ~~was detected~~. In contrast, all the cysts developed in mice treated with ABZ-LNCs or ABZ suspension revealed changes in the germinal layer~~. A~~ with a reduced number of germinal cells ~~were~~ being detected in cysts recovered from the ABZ suspension-treated group (Fig. 5.b). The damage extension appeared to be greater after ABZ-LNC~~s~~. The germinal layer was extensively distorted, ~~where only debris of cells treatment,~~ as only cell debris could be observed (Fig. 5.c).

[Figure 5]

DISCUSSION

CE is a neglected disease, especially in developing countries, ~~with~~ which has had an increasing economic impact due to the need for livelong treatments (Narra et al., 2016). ~~The~~ Radical resection of the cyst mass represents the traditional treatment strategy, ~~and is, in many instances,~~

accompanied in many cases by chemotherapy. For inoperable cases, ABZ is considered to be a cornerstone pharmacological treatment.

~~Nevertheless,~~ Hydrophobicity is an important physicochemical parameter of ~~this~~ drugs which determines the rate and degree of absorption. In the case of ABZ, its slow dissolution rate limits ~~thus limiting~~ the production of highly potent ABZ formulations (Teggi, 2004). ~~Nanodrug delivery systems have been widely reviewed for their use in several formulations to~~ To try to improve drug bioavailability, prolonged drug release and ~~decrease~~ minimize side effects of many drug candidates (Allam et al., 2017)~~.~~, nanosized drug delivery systems have been widely reviewed for different formulations. Regarding this, particle size and size distribution seem~~s~~ to be ~~one~~ two of the most important characteristics related to ~~their~~ biodistribution properties. Indeed, the key feature is ~~its~~ an enlarged surface area, which ~~allows~~ improves dispersion in aqueous environments and leads to a faster saturation in the dissolution layer around the particles with a consequent increase in dissolution velocity (Murdande et al., 2015). Different strategies have been developed in order to improve ABZ water solubility and its dissolution rate, such as preparation of a self-microemulsifying drug delivery system (Mukherjee and Plakogiannis, 2010), its incorporation into liposomes (Lv et al., 2013; Panwar et al., 2010) and its complexation with cyclodextrins (García et al., 2014; Pradines et al., 2014). ~~Alternatively~~ In addition, ABZ solubility has been improved by preparing hot-melt extrusion formulations with polyvinylpyrrolidone (Hengsawas Surasarang et al., 2016; Martinez-Marcos et al., 2016) and solid lipid nanoparticles (Ahmadnia et al., 2013). ~~Although the~~ However, preparation methods which include~~d~~ the use of rotatory evaporators and organic solvents, as well as high ~~quantity/~~concentrations of complexing agents are limiting factors in the application of these formulations.

LNCs are nanocarriers produced by a simple phase temperature inversion process without the use of organic solvents and are able to entrap many hydrophobic drugs such as ABZ. The structure of this vector is unique~~,~~ and it is composed of an oily core, in which the drug is solubilised, and

surrounded by a shell ~~composed~~ made of lecithin and polyethylene-glycol chains (Anton and Saulnier, 2013; Anton et al., 2007; Huynh et al., 2009). In the present study ~~this work~~, we have fully characterised the ABZ-LNC~~s~~ formulations previously described (Pensel et al., 2015)~~.~~ ~~We achieved~~ and attained the encapsulation of ABZ (EE% ≥90%) in a stable LNC~~s~~ formulation with a mean particle size around 50 nm and an acceptable PI which demonstrates the monodispersity of this preparation.

Concerning the administration of nanoparticles by the oral route, several biopharmaceutical parameters should be considered in order to obtain a good efficacy/safety ratio. The first barrier to overcome after oral administration is ~~constituted by~~ the physicochemical environment of the gastrointestinal tract. It is known that nanoparticles are susceptible to aggregation in media with a high ionic strength, extreme pH or high enzyme/protein content, and thus, the surface composition of the nanocarrier plays an important role in its stability (Prego et al., 2006). In this study, we demonstrated the stability of these nanocapsules in both ~~either~~ the gastric and intestinal media. The positive effect of the Kolliphor® HS-15 coating used, ~~consisted on~~ was in reducing the number of interactions of particles with the digestive enzymes and this is in ~~accordance~~ agreement with the PEG stabilizing effects previously reported by Tobío et al., 2000. Moreover, the stability study (Fig. 2) also revealed no burst release of ABZ loaded LNCs, so that the drug transport out of the LNCs ~~would be~~ may have been driven mainly by a diffusion-controlled mechanism (Roger et al., 2017). In consequence, ABZ ~~could be~~ was released for a prolonged period of time from these nanocapsules ~~means~~ implying a longer residence time in systemic circulation, which ~~could~~ consequently can improve the delivery to target tissues.

In a previous ~~work~~ study, we characterized the plasma and cyst drug exposure after the administration of ABZ loaded LNCs in mice infected with *E. granulosus* (Pensel et al., 2015). More enhanced albendazole sulfoxide (ABZSO) concentration profiles were obtained in plasma and cysts from ABZ-LNC~~s~~ orally and subcutaneously treated animals ~~both orally and subcutaneously~~,

compared ~~to~~ with those observed after oral administration of ABZ suspension. Additionally, the ABZSO concentrations measured in cysts from ABZ-LNC~~s~~ treated mice were 1.7-fold higher than those detected in plasma, ~~. The~~ with this capacity of LNCs to increase oral bioavailability of ABZ ~~could be~~ possibly being explained in part, by the gastrointestinal stability of the particles ~~reveled~~ observed in this investigation.

~~In the present work~~ The chemoprophylactic efficacy of our formulation was evaluated in our study by ~~.~~ ~~This study~~ simulating a cyst rupture during surgical practice and ~~the~~ concomitant drug treatment. ~~Indeed,~~ This is the first report of nanoparticles loaded with ABZ ~~which~~ improving the chemoprophylactic efficacy of the drug in mice infected with *E. granulosus*. Interestingly, ABZ loaded in LNCs ~~have~~ produced a greater preventive effect than ABZ alone, since ~~not only~~ this reduced not only the number of developed cysts, but may also have inhibited the development of hydatid cysts in mice.

Metacestodes are fluid-filled vesicles that are separated into: (i) an inner germinal layer representing the living and metabolically active parasite tissue, and (ii) an outer, acellular compartment known as the laminated layer, which mediates the direct physical contact with the host immune and non-immune cells (Shuhua et al., 2002). As ~~it is seen~~ can be observed in figure 5 ~~6~~, only cells debris ~~of cells could be observed for~~ was present in ABZ-LNCs treatments with a marked alteration in the germinal layer ~~with internal tissue~~ ~~extensively distorted, internal tissue, vacuolated areas and the presence of lamellar bodies~~. ~~Altogether~~ The improved ~~in the~~ therapeutic efficacy of ABZ-LNCs ~~previously demonstrated~~ could be due to ~~the~~ an increased oral bioavailability of ABZ due to the enhancement of its permeability across the intestine and/or a decrease of the intestinal metabolism. However, more specific studies should be performed to get more detailed information on the host-parasite interactions that occur during drug treatments.

5. Conclusion

In conclusion, this study reports on the characterisation of a novel carrier based on the incorporation of an antiparasitic drug into LNCs and describes their potential use ~~as~~ in CE treatment ~~was reported~~. These nanocarriers exhibited ~~attractive~~ adequate properties in terms of size, drug payload and physicochemical stability which make them an interesting alternative for the oral delivery of ABZ. In addition, ABZ-LNCs ~~showed~~ revealed a higher chemoprophylactic efficacy in comparison to ABZ suspension administered by the oral route. Our results ~~complement and reinforce~~ are in agreement with the clinical efficacy, ~~and the~~ observed in previous pharmacokinetics studies ~~reported before in order to elucidate~~ concerning the mechanism of action of this carrier and its potential use as a drug delivery system for CE treatment in humans.

CONFLICT OF INTEREST STATEMENT

The authors have declared that no conflict of interest exists

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Fig.1.a.b. SEM micrographs of ABZ-LNCs (from batch B in table 1).

Fig.2. Encapsulation efficiency (EE%) of ABZ in LNCs following incubation in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) with enzymes (Mean± SD.; n=3).

Fig.3. Stability determination of ABZ-LNCs following incubation in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) with enzymes (Mean± SD.; n=3). a) mean particle size, b) polydispersity index (PI).

Fig.4. Box Plot: Chemoprophylactic efficacy study. Mean (±SD) weights (g) of hydatid cysts recovered six months post-infection ~~of~~ from mice ~~from~~ of control groups (water and blank LNCs) and treated groups (ABZ-LNCs and ABZ suspension). Treatments were given ~~at~~ of 5 mg/kg, every 24 h ~~over~~ for 30 days following infection. The different letters indicate statistically significant differences ($P<0.05$) between experimental groups.

Fig.5. Representative SEM images of hydatid cysts recovered from infected mice during the chemoprophylactic efficacy study. a) Cysts from unmedicated animals (gl: germinal layer; ×800). b) Cysts recovered from mice treated with ABZ-suspension (5 mg/kg). Alteration of the germinal layer (gl) can be ~~appreciated, where~~ observed with only a few cells exhibiting an intact morphology (×800). c) Cysts recovered from mice treated with ABZ-LNCs (5mg/kg). The germinal layer is altered ~~and~~ with only cell debris ~~of cells can be~~ being observed (×700).

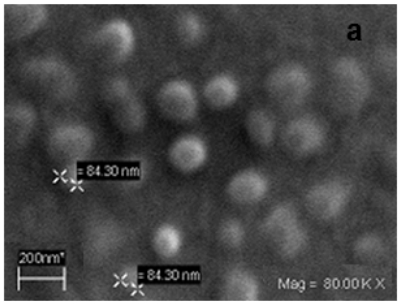
Table 1. Physical-chemical characterisation of different oil core ABZ-LNCs formulations. (Mean± SD.; n=3; PI= polydispersity index).

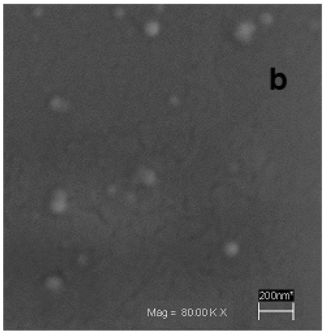
	FORMULATIONS			
	A*	B*	C*	Blank LNCs**
Size (nm)	56.2±0.2	47.9±1.1	54.2±0.9	49.56±0.74
PI	0.08	0.08	0.09	0.07
Zeta potential (mV)	-16±2	-18±3	-17±3	-17±2.4
Encapsulation efficiency (%)	77±2	97.5±1.8	75±4	-
Loading (mg/g)	0.22±0.01	0.28±0.002	0.21±0.003	-

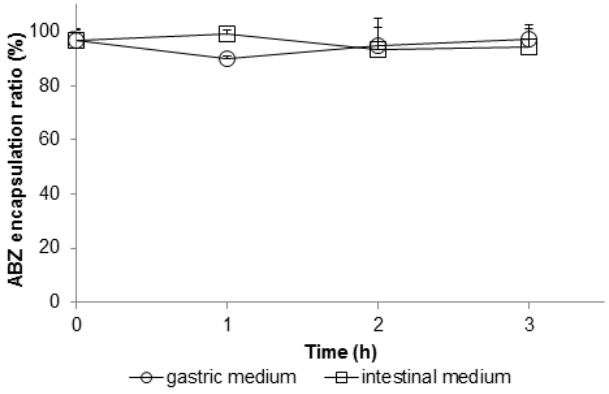
*Lipid matrix: A) Labrafac® + OA; B) Captex®8000 +OA C) Labrafac®/Captex®8000 (50/50) + OA.
** Blank LNCs were made as a reference with Captex®8000+OA as the oily core.

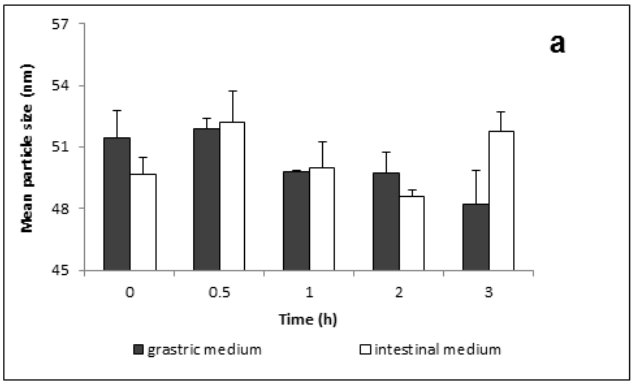
Table 2. Storage stability. Characterization in terms of size, zeta potential (ZP), encapsulation efficiency (EE%) and drug payload of LNCs containing ABZ after 60 days at 4-8 ºC. (Mean ± S.D.; n=3). P.I: Polydispersity Index.

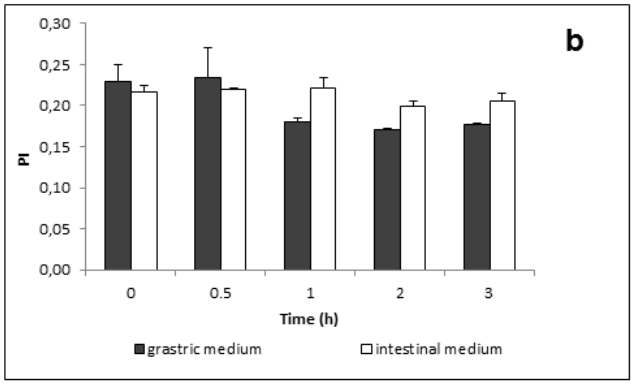
	EE%	Drug payload (mg/g LNCs)	Size (nm)	PI	ZP (mV)
0 days	97.5±1.9	0.28±0.05	46.1±1.2	0.08	-14.8±1.8
60 days	91.1±1.7	0.26±0.05	46.2±2.7	0.10	-14.0±1.9







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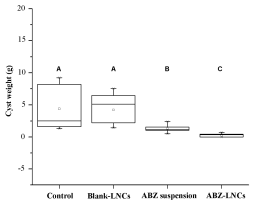

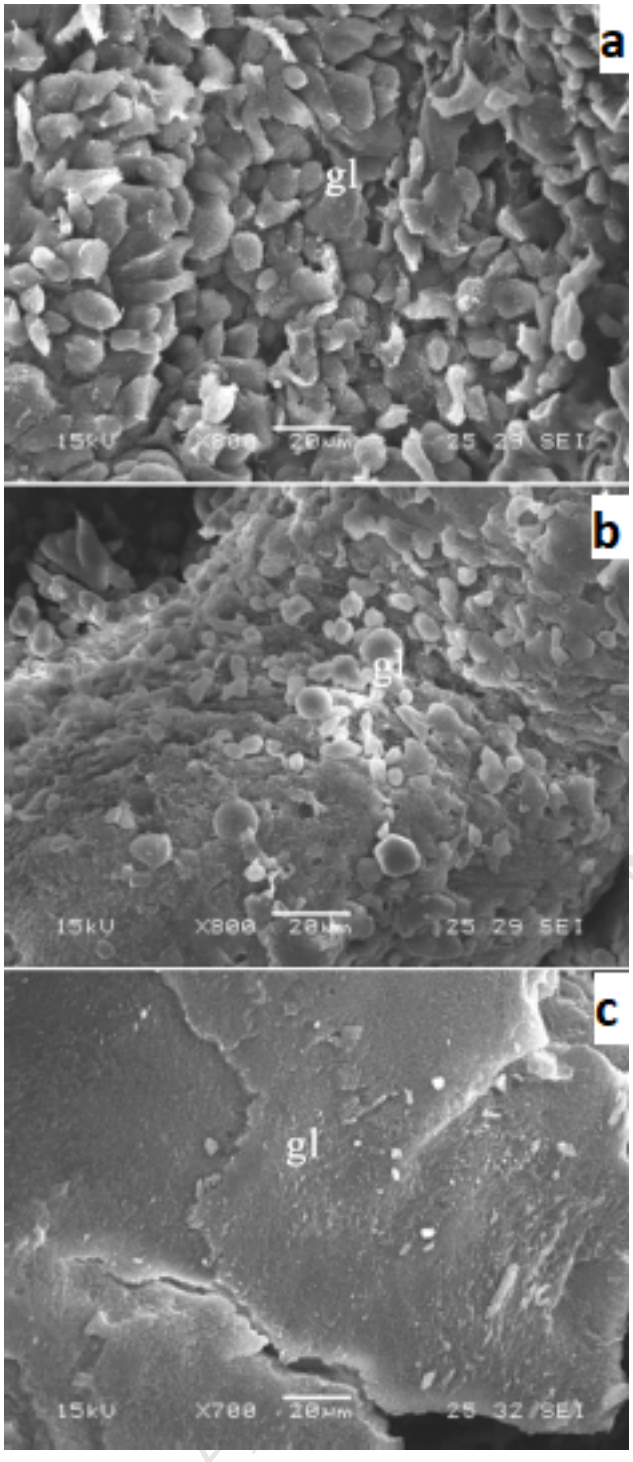

HIGHLIGHTS

- Optimised ABZ-LNCs were obtained through the phase inversion method
- ABZ-LNCs with a EE >90%~~)~~ were stable for at least 2 months
- ABZ remained encapsulated in LNCs after its incubation in simulated biological fluids
- ABZ-LNCs showed a greater chemoprophylactic efficacy than ABZ suspension
- ABZ-LNCs could be a promising strategy for ~~in the~~ cystic echinococcosis treatment