Drug repurposing for the treatment of alveolar echinococcosis: *in vitro* and *in vivo* effects of silica nanoparticles modified with dichlorophen

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Running title: Silica nanoparticles modified with dichlorophen against *Echinococcus multilocularis*.

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Abstract

Alveolar echinococcosis is a neglected parasitic zoonosis caused by the metacestode *Echinococcus multilocularis*, which grows as a malignant tumor-like infection in the liver of humans. Albendazole is the antiparasitic drug of choice for the treatment of the disease. However, its effectiveness is low, due to its poor absorption from the gastro-intestinal tract. It is also parasitostatic and in some cases produces side effects. Therefore, an alternative to the treatment of this severe human disease is necessary. In this context, the repositioning of drugs combined with nanotechnology to improve the bioavailability of drugs, emerges as an useful, fast, and inexpensive tool for the treatment of neglected diseases. The *in vitro* and *in vivo* efficacy of dichlorophen (DCP), an antiparasitic agent for intestinal parasites, and silica nanoparticles modified with DCP (NP-DCP) was evaluated against *E. multilocularis* larval stage. Both formulations showed a time and dose-dependent *in vitro* effect against protoscoleces. The NP-DCP had a greater *in vitro* efficacy than the drug alone or albendazole. *In vivo* studies demonstrated that the NP-DCP (4 mg/kg) had similar efficacy to ABZ (25 mg/kg) and greater activity than the free DCP. Therefore, the repurposing of DCP combined with silica nanoparticles could be an alternative for the treatment of echinococcosis.

Key words

Alveolar echinococcosis; *Echinococcus multilocularis*; albendazole; dichlorophen; silica nanoparticles.

Introduction

Alveolar echinococcosis (AE), caused by the metacestode stage of the fox tapeworm *Echinococcus multilocularis*, represents one of the most severe parasitic zoonosis with endemic areas in the Northern hemisphere (Deplazes *et al*., 2017). The life cycle of *E. multilocularis* is perpetuated between canid definitive hosts such as foxes (*Vulpes vulpes*) and small rodents (mainly voles) as natural intermediate hosts. Humans can accidentally acquire the infection through ingestion of eggs shed in the feces of a definitive host. Metacestode tissue grows like a malignant tumor-like lesion into the surrounding liver tissue and can infiltrate adjacent organs and tissues producing distant metastases. If not appropriately treated, parasite expansion will eventually lead to organ failure and death (Kern *et al*., 2017).

The current strategies for treating human AE are surgical resection of parasite lesions accompanied by chemotherapy with benzimidazoles (BMZ) carbamate derivatives, and for inoperable cases, chemotherapy alone is the only option. Albendazole (ABZ) is the most common and effective antiparasitic drug for AE treatment. Alternatively, mebendazole may be used if ABZ is not available or not tolerated (Siles-Lucas *et al*., 2018). However, after treatment interruption, the recurrence of the disease is commonly reported as the effect of BMZ is parasitostatic rather than parasitocidal (Kern *et al*., 2017). Moreover, after its oral administration, ABZ shows erratic absorption from the gastrointestinal tract leading to low drug levels in plasma. Consequently, ABZ often has to be administered life-long at higher doses causing severe side effects in some patients (Daniel- Mwambete *et al*., 2004). To overcome such problems, it is necessary to develop new chemotherapeutic alternatives for the treatment of AE that provide greater efficacy and bioavailability, low toxicity and reduced side effects.

Drug repurposing is a strategy that accelerates the drug development process as a result of reducing costs and risks and decreasing time to market (Baker *et al*., 2018). Important drug repurposing efforts have been recently directed against several helminth infections (Panic *et al*., 2014). Particularly in the experimental chemotherapy of AE, a variety of anticancer, antifungal and antiprotozoal drugs have demonstrated *in vitro* effects against the larval stage of *E. multilocularis* (Lundström-Stadelmann *et al*., 2019). However, only a few of these compounds showed efficacy in infected mice and only mefloquine, and amphotericin B alone or combined with nitazoxanide were assayed in humans (Siles-Lucas *et al*., 2018).

Dichlorophene (DCP) is a halogenated phenolic compound used as bactericide and fungicide in cosmetic product formulations (Yamarik, 2004). It was evaluated *in vitro* and *in vivo* as an anthelmintic drug. *In vitro* studies include the use of DCP on *Hymenolepis nana* (Sen and Hawking, 1960), *E. multilocularis* (Sakamoto, 1973), *Taenia saginata* (Grinenko, 1964), and *Trichostrongylus colubriformis* (Rapson *et al*., 1985). Several *in vivo* studies have been conducted with DCP against *Anoplocephala perfoliata* (Fukui *et al*., 1960), *Moniezia expansa*, *M. benedeni* (Fukui, 1960), *Ancylostoma caninum* (Miller, 1966), and *E. granulosus* (Gemmell, 1958). DCP is used as a narrow-spectrum cestocide for treatment of dogs and cats against *Taenia* spp. (Maddison *et al*., 2008). Moreover, DCP has been assessed in humans to lower the parasite burden of the intestinal tapeworms *T. saginata*, *H. nana*, and *Ascaris lumbricoides* (Jackson, 1956; Adams and Seaton, 1959; Biagi *et al*., 1959).

However, DCP has a very low aqueous solubility and is poorly absorbed after oral administration (Maddison *et al*., 2008). In this context, the development of nanodevices for drug delivery offers new treatment options that improve the solubility, the absorption and minimize secondary effects and toxicity (Irache *et al*., 2011). Nanoparticles are defined as particles with size

in the range of 1 to 100 nm at least in one of the three dimensions. They can be classified into organic and inorganic nanoparticles, according to their material properties. The latter group includes silica nanoparticles (Llinàs and Sánchez-García, 2014). Drugs absorbed into silica nanoparticles were used against microorganisms. Higher efficacy of modified nanoparticles with organic drugs in relation to the drug alone has been reported (Arce *et al*., 2012). The aim of the present work was to evaluate the *in vitro* and *in vivo* efficacy of DCP and silica nanoparticles modified with DCP (NP-DCP) against *E. multilocularis* larval stage.

Materials and methods

Synthesis and characterization of the nanoparticles modified with dichlorophen

The NP-DCP were prepared according to the method reported by Escalada *et al*. (2014). Briefly, 0.5 g of DCP and 180 ml of o-xylene were added to 1.0 g of silica nanoparticles. The mixtures were placed in a Soxhlet extractor containing CaH_2 equipped with a condenser with anhydrous $CaCl_2$, and refluxed during 24 h. The products were filtered with 20 nm-nylon filters, washed with 50 ml hot o-xylene and finally with 50 ml ethyl acetate. The resulting gel was first dried at 0.1 Torr and at room temperature for 3 h and then at 120 ºC for 5 h. White powders were obtained.

The characterization was realized according to Escalada *et al*. (2014). Comparison of attenuated total reflection infrared (ATR-IR) spectra of NP-DCP and DCP showed that the organic drug was covalently bonded to the silica nanoparticles. This result was further confirmed by UV-visible spectroscopy and by thermogravimetry. From this technique, the percentage of DCP present in NP-DCP was 5 %.

Parasite material of E. multilocularis

All experiments were carried out using *E. multilocularis* isolated J2012 (kindly provided by Klaus Brehm, Institute for Hygiene and Microbiology, University of Würzburg, Germany). The parasite was propagated in the peritoneum of CF-1 mice and was processed as described by Albani *et al*. (2015), with some modifications. Briefly, the parasitic suspension obtained after cutting the metacestodes and passing them through a metallic strainer was washed several times with phosphate-buffered saline (PBS) 1x. Finally, 0.5 vol of PBS 1x and 6 µl/ml of ciprofloxacin (2 mg/ml) were added to parasite tissue and incubated overnight at 4 ºC.

In vitro *experiments*

Isolation and in vitro *culture of protoscoleces of* E. multilocularis

From the homogenized metacestode material, the protoscoleces were isolated according to Albani and Elissondo (2014). Briefly, the parasite material was vigorously shaken and then passed through a polyester gauze (pore size 150 µm). The filtered material was recovered and finally passed through a second polyester gauze (pore size 30 µm). The material that was retained in the gauze contains the protoscoleces, which were cultivated (2000 viable and free protoscoleces per Leighton tube) in 10 ml of medium 199 (Lab. Microvet S.R.L., Argentina) supplemented with 100 µg/ml streptomycin, 60 µg/ml penicillin, 50 µg/ml gentamicin and 4 mg/ml glucose. Cultures were performed at 37 ºC with changes of culture medium every 5 days.

In vitro *cultivation of* E. multilocularis *metacestode vesicles*

From the fresh parasitic material of *E. multilocularis*, the metacestode vesicles were obtained following the procedure established by Spiliotis and Brehm (2009). Briefly, a co-culture was performed between the parasitic material (1 ml) and 10^6 rat hepatocytes RH- (Reuber hepatoma cells: ATCC No. CRL-1600) in 50 ml of 199 medium, supplemented with 4,5 g/l glucose, 10% fetal bovine serum (FBS), 100 µg/ml streptomycin and 60 µg/ml penicillin. The culture was performed in 75 cm^2 culture flasks at 37 ºC, 5 % CO_2. Medium was changed once a week and after that, 10^6 RH- cells were added. Vesicles between 2-5 mm in diameter were used for the assays.

Metacestode vesicles were cultured in 24 well plates (10 vesicles per well), with 2 ml/well of 199 medium with FBS and antibiotics, and incubated at 37 ºC, 5 % CO_2, without changes of culture medium.

In vitro *drug treatment*

Silica nanoparticles and NP-DCP were suspended in medium 199 supplemented with antibiotics at a drug concentration of 0.2 mg/ml. DCP (Sigma-Aldrich) was dissolved in dimethyl sulfoxide (DMSO) at drug concentrations of 0.5 and 0.1 mg/ml and ABZ (Sigma Aldrich) was also dissolved in DMSO to obtain a drug concentration of 10 mg/ml. In each case, the drugs were added to the medium at the final concentrations tested.

Experimental design and evaluation of in vitro *efficacy of DCP and NP-DCP*

Protoscoleces of E. multilocularis

The *in vitro* efficacy of different concentrations of DCP and NP-DCP (1, 0.5 and 0.1 μg/ml) against protoscoleces of *E. multilocularis* was evaluated. Protoscoleces incubated in culture medium 199 with 1 μl of DMSO, with silica nanoparticles (1 μg/ml) and with ABZ (10 μg/ml) were used as controls. Each experiment was performed in triplicate and was repeated 3 times.

Daily, culture tubes were followed microscopically to determine the appearance of morphological alterations. Every two days, 90-100 protoscoleces were taken from each of the tubes for viability assessment using the methylene blue exclusion test. Samples of protoscoleces for scanning electron microscopy (SEM) were taken after 2 and 6 days of incubation.

Metacestode vesicles of E. multilocularis

The *in vitro* efficacy of different concentrations of DCP and NP-DCP (1, 0.5 and 0.1 μg/ml) against metacestode vesicles of *E. multilocularis* was evaluated. Metacestodes incubated in culture medium 199 with 1 μl of DMSO and with ABZ (10 μg/ml) were used as controls. Each experiment was performed in triplicate.

Culture plates were followed microscopically every day to determine the appearance of cysts with collapse of the germinal layer. This parameter was used as criteria of metacestode vesicles viability.

In vivo *experiments*

In vivo *drug treatment*

For *in vivo* efficacy studies against the murine model of AE, ABZ suspension (3.33 mg/ml) was prepared by dissolution of ABZ pure standard in distilled and deionized water (pH 7.0) and shaking for 12 h. Also, a solution of DCP (0.53 mg/ml) and a suspension of NP-DCP (4 mg/ml) were prepared in distilled and deionized water by shaking for 24 h. All formulations were vigorously shaken before administering to mice.

Experimental design and evaluation of in vivo *efficacy of DCP and NP-DCP against the murine model of AE*

For the therapeutic and chemoprophylactic efficacy studies, female CF-1 mice (n = 50 for each study) were intraperitoneally infected with 0.3 ml of homogenized parasitic material of *E. multilocularis*. In the chemoprophylactic efficacy study, the treatment of the animals began 1 day post-infection and in the therapeutic efficacy study the dosage of mice began 6 weeks post-infection. In both studies, treatments were performed daily for 30 days by intragastric administration. The experimental groups (10 animals/group) were: a- water control group, mice received distilled and deionized water as a placebo; b- NPs control group, animals received a suspension of silica nanoparticles in deionized water; c- ABZ group, mice received ABZ suspension (25 mg/kg); d- DCP group, mice were treated with a solution of DCP (4 mg/kg); e- NP-DCP group, animals received a NP-DCP suspension (4 mg/kg).

At the end of the *in vivo* studies (approximately 10 weeks post-infection), the animals were euthanized and the necropsy was carried out immediately thereafter, and the cysts mass were recovered from the peritoneal cavity. As described by Albani *et al*. (2015), the efficacy of the

treatments was determined by the mean cysts weight from each group, the viability of protoscoleces obtained from each group (as described above in *Experimental design and evaluation of* in vitro *efficacy of DCP and NP-DCP* section) and by the ultrastructural features of cysts and protoscoleces recovered from mice by SEM.

Scanning electron microscopy

Samples of protoscoleces cultured *in vitro* as well as protoscoleces and cysts recovered from mice involved in both *in vivo* efficacy studies were processed for SEM as described by Elissondo *et al*. (2006, 2007).

Statistical analysis

The results obtained from *in vitro* and *in vivo* studies are presented as mean ± standard deviation (SD). To compare the survival of protoscoleces and metacestode vesicles exposed to different concentrations of DCP and NP-DCP, the log-rank test was used, performed with the software BioEstat 5.0 (Ayres *et al*., 2007). Cysts weights and protoscoleces viability from *in vivo* assays were compared by Kruskal Wallis Test (nonparametric method) followed by Dunn´s Multiple Comparisons Test. The analysis was carried out using Instat 3.0 software program (GraphPad Software, San Diego, CA, USA). In all cases, P values less than 0.05 ($P < 0.05$) were considered statistically significant.

Results

Evaluation of in vitro *efficacy of DCP and NP-DCP against* E. multilocularis *protoscoleces*

Figure 1 shows the survival of protoscoleces of *E. multilocularis* after the *in vitro* exposure to 10 μg/ml of ABZ and to different concentrations of DCP and NP-DCP (1, 0.5 and 0.1 μg/ml). Control protoscoleces remained viable throughout the experimental period and no changes in structure (Fig. 2a) and ultrastructure (Fig. 3a) were observed. Silica nanoparticles (data not shown) and ABZ did not show significant differences compared to DMSO-medium group ($P = 0.4698$ and $P = 0.3505$, respectively).

DCP and NP-DCP produced time and dose-dependent protoscolicidal effect ($P < 0.0001$). Treatment of protoscoleces with both drugs caused a reduction of larval viability. However, NP-DCP had a stronger protoscolicidal effect compared to DCP.

The greatest protoscolicidal effect was observed with the concentration of 1 μg/ml of NP-DCP, causing a rapid decrease in viability and reaching 0 % after 6 days. NP-DCP at a concentration of 0.5 μg/ml also generated a rapid decrease in the viability of protoscoleces, reaching 0 % after 10 days. The concentration of 0.1 μg/ml of NP-DCP reduced the viability to 19.67 ± 13.3 % at the end of the assay (Fig. 1).

The treatment of protoscoleces with 1 μg/ml of DCP caused a marked decrease in their survival after 4 days. Then, the viability decreased slowly. The protoscolicidal effect observed with the concentration of 0.5 μg/ml of DCP was similar to that caused by 0.1 μg/ml of NP-DCP ($P = 0.1377$). The decrease in viability was slow and gradual. In addition, 0.1 μg/ml of DCP did not show differences compared to DMSO-medium group ($P = 0.6316$).

At 2 days p.i., the protoscoleces treated with 1 μg/ml of NP-DCP showed total loss of their morphology (Figs. 2d and 3f) and the concentration of 0.5 μg/ml caused a total loss of microtriches

and hooks, with the presence of blebs in the tegument (Figs. 2e and 3g). Protoscoleces treated with 0.1 μg/ml of NP-DCP at 2 days p.i. showed alteration of tegument of the soma region (Fig. 3h) and the appearance of blebs in the tegument was observed after 4 days (Fig. 2f).

At day 2 p.i. the protoscoleces exposed to 1 μg/ml of DCP showed blebs in the tegument, in addition to the loss of rostellar hooks (not shown) and contraction of soma (Figs. 2c). However, after 6 days p.i., loss of microtriches and alteration of the tegument with the presence of small blebs were observed (Fig. 3c). Protoscoleces incubated with 0.5 μg/ml of DCP for 2 days did not present ultrastructural alterations. After 4 days, the appearance of blebs in the tegument was detected (data not shown). Protoscoleces incubated for 6 days showed loss of microtriches and blebs in the tegument (Fig. 3d). Finally, DCP at 0.1 μg/ml produced soma contraction and alteration of the tegument of the soma region after 6 days p.i. (Fig. 3e). These alterations were similar to the changes caused by 10 μg/ml of ABZ (Fig. 2b and 3b).

Evaluation of in vitro *efficacy of DCP and NP-DCP against metacestode vesicles of* E. multilocularis

Figure 4 shows the survival of metacestode vesicles after *in vitro* incubation for 3 and 10 days with different concentrations of DCP and NP-DCP. The viability of control vesicles was 100 % throughout the entire assay (Fig. 5a), as well as metacestodes incubated with 10 μg/ml of ABZ and 0.1 μg/ml of DCP. Treatments with 1 and 0.5 μg/ml of DCP and with 0.1 μg/ml of NP-DCP produced a decrease in the viability of metacestodes. However, no significant differences were found with respect to the control ($P = 0.24$, $P = 0.22$, $P = 0.68$, respectively; Fig. 5d). NP-DCP at the concentrations of 1 and 0.5 μg/ml were the only treatments that had a significant effect on the

viability of vesicles ($P < 0.0001$) causing the collapse of the germinal layer of metacestode vesicles after 1 and 2 days of incubation, respectively (Figs. 5b and 5c).

Therapeutic efficacy study of DCP and NP-DCP against the murine model of AE

All mice belonging to the therapeutic efficacy study developed cysts in the peritoneal cavity. There were no significant differences ($P > 0.05$) between the weight of the cysts of both control groups. However, oral administration of 25 mg/kg of ABZ and 4 mg/kg of NP-DCP caused a significant decrease ($P < 0.05$) in the mean weight of the cysts compared to control groups. The treatment with 4 mg/kg of DCP did not cause a decrease in the weight of the developed cysts (Table 1).

The ultrastructural study by SEM of the germinal layer of metacestodes recovered from control and treated groups is shown in Figure 6. The germinal layer of cysts obtained from control mice showed the characteristic multicellular structure (Fig. 6a). In contrast, metacestodes developed in mice treated with 25 mg/kg of ABZ and with 4 mg/kg of DCP showed areas without cells and the presence of altered cells in germinal layer (Fig. 6b, 6c and 6d). However, in the germinal layer of cysts recovered from NP-DCP-treated mice greater extensions of areas without cells as well as altered cells were evidenced (Fig. 6e).

Figure 7A shows the viability of protoscoleces obtained from the different experimental groups of the therapeutic efficacy study. The protoscoleces isolated from the control groups had a viability greater than 80 % and no changes in ultrastructure were observed (Fig. 7Ba). The treatment with 25 mg/kg of ABZ and with 4 mg/kg of NP-DCP showed protoscolicidal effect, reducing significantly the viability of protoscoleces ($P < 0.05$) to 23.92 ± 29.0 % and 28.67 ± 25.2

%, respectively. The administration of 4 mg/kg of DCP reduced the survival of protoscoleces to 36.90 ± 30.8%, but it was not significant with respect to control groups.

The analysis of the images of protoscoleces by SEM was consistent with the viability data obtained (Fig. 7B). Most of the protoscoleces obtained from mice of the ABZ and NP-DCP groups showed a total loss of morphology (Figs. 7Bb and 7Bd), while protoscoleces obtained from DCP group showed only some alterations, such as loss of hooks (Fig. 7Bc).

Chemoprophylactic efficacy study of DCP and NP-DCP against the murine model of AE

Cysts development in the peritoneal cavity was observed in all mice belonging to chemoprophylactic efficacy study. No significant differences were found ($P > 0.05$) between the mean weight of the cysts of the control water and control NPs groups. The treatments with DCP and NP-DCP did not significantly reduce the weight of the cysts, while, administration of ABZ produced a significant decrease compared to the weight of cysts from the water control group ($P < 0.05$; Table 1).

Metacestodes recovered from the different treatments lost their characteristic multicellular structure. The treatment with ABZ caused a decrease in the number of cells (Fig. 8b) and the presence of altered cells. Although the metacestodes recovered from the mice treated with DCP caused a reduction in cell number of the germinal layer (Fig. 8c), the extension of the damage was lower than with NP-DCP treatment, where only a few cells were observed (Fig. 8d).

The viability of protoscoleces of the control groups was greater than 90 % (Fig. 9A). The treatment of the mice with 4 mg/kg of DCP did not reduce the viability of the protoscoleces with respect to the control groups. Protoscoleces recovered from the mice treated with 25 mg/kg of

ABZ (36.42 ± 30.0 %) and with 4 mg/kg of NP-DCP (57.28 ± 22.9 %) showed a significant decrease of the viability compared to the control groups ($P < 0.01$).

The ultrastructural analysis of protoscoleces coincided with the percentages of viability obtained from the different experimental groups. The protoscoleces of the control groups were observed unchanged (Fig. 9Ba and 9Bb), as well as those obtained from the DCP group (Fig. 9Bd). In contrast, protoscoleces isolated from the cystic masses belonging to the mice of the groups ABZ and NP-DCP presented alterations. The protoscoleces of the ABZ group showed rostellar disorganization and contraction of the soma (Fig. 9Bc). The protoscoleces of the NP-DCP group evidenced loss of hooks and microtriches and the presence of vesicles in the tegument (Fig. 9Be and 9Bf).

Discussion

A perfect agent for the chemotherapeutic treatment of echinococcosis should be selectively toxic for the cestode and present a favorable solubility and absorption to reach a therapeutic concentration in the target site. The lack of undesirable clinical effects and a parasitocidal activity are also desirable (Siles-Lucas *et al*., 2018).

Chemotherapeutic treatment of AE with BMZ has several disadvantages, such as variations in treatment success due to the low and erratic bioavailability of the antiparasitic agent after oral administration, the adverse reactions observed in patients after prolonged treatments and a parasitostatic rather than a parasitocidal effect (Daniel-Mwambete *et al*., 2004; Hemphill *et al*., 2014; Grüner *et al*., 2017; Kern *et al*., 2017).

In this context, the drug repurposing approach is particularly important for neglected tropical diseases. The time, effort and resources saved in the reuse of already approved drugs with known physicochemical, pharmacokinetic and toxicological properties make this approach an intelligent and ethical option (Panic *et al*., 2014). Consequently, research about novel compounds for the treatment of echinococcosis should focus on drugs or classes of drugs that are already marketed and/or in clinical development (Küster *et al*., 2015).

Considerable efforts have been made to improve the chemotherapeutic treatment of AE. Hundreds of anti-infective agents, anti-cancer drugs and antiparasitic compounds were *in vitro* tested against the larval stage of *E. multilocularis* (Siles-Lucas *et al*., 2018; Lundström-Stadelmann *et al*., 2019). Most of these repurposing initiatives had poor *in vivo* results. Nitazoxanide (Stettler *et al*., 2004) and mefloquine (Küster *et al*., 2015; Rufener *et al*., 2018) showed efficacy against the murine model of AE. On the other hand, amphotericin B alone or combined with nitazoxanide were the only drugs tested in human patients as an alternative to the treatment with ABZ (Reuter *et al*., 2003; Tappe *et al*., 2009).

DCP has demonstrated anthelmintic *in vitro* and *in vivo* efficacy, mainly against intestinal parasites. The only study against a systemic parasitic infection was carried out *in vitro* against the larval stage of *E. multilocularis* (Sakamoto, 1973). However, the low solubility of DCP in water limits its activity against systemic parasitic diseases. Drug repurposing complemented by nanotechnological strategies could be a useful tool to improve the bioavailability and efficacy of repurposed drugs (El-Moslemany *et al*., 2016). In this context, we evaluated the *in vitro* and *in vivo* efficacy of DCP and NP-DCP against the larval stage of *E. multilocularis*.

The *in vitro* activity of DCP against *E. multilocularis* protoscoleces was previously demonstrated by Sakamoto (1973). Although this author used concentrations of DCP 10-100 times

higher than those evaluated in this work, the viability results were similar. During our *in vitro* studies against protoscoleces, both DCP formulations showed time and dose-dependent effect. However, NP-DCP had a stronger effect compared to DCP. The NP-DCP at a concentration of 1 µg/ml reduced the viability of protoscoleces to 0 % after 4 days. In contrast, 10 % of the protoscoleces incubated with the same concentration of DCP were alive at the end of the assay. These results are consistent with the observations reported by Vico *et al*. (2016). The authors demonstrated an increase in the antimicrobial activity of silica nanoparticles functionalized with gallic acid against *Paenibacillus larvae* in comparison to free gallic acid.

The results of the viability test coincide with the structural and ultrastructural alterations observed by light microscopy and SEM. Moreover, the ultrastructural alterations agreed to those reported for *E. multilocularis* protoscoleces incubated with ABZ, thymol, and the combination of both drugs (Albani and Elissondo, 2014).

Only the NP-DCP caused a significant decrease of the viability of the *E. multilocularis* metacestodes during the *in vitro* assay. Moreover, the activity of the NP-DCP against protoscoleces and cysts was higher than the effect induced by ABZ. This could be explained because the anthelmintic activity of BMZ compounds not only depend on its binding to parasite ß tubulin but also on their ability to reach high and sustained concentrations within the parasite cells, in sufficient time, to cause the therapeutic effect (Thompson and Geary, 1995).

The activity of DCP and NP-DCP was also evaluated in an *in vivo* murine model of AE. The therapeutic and the chemoprophylactic efficacy of both formulations of DCP were evaluated on mice infected with *E. multilocularis*. The LD50 value for DCP was 1670 mg/kg orally (Yamarik, 2004). This dose is more than 400 times higher than the dose used in this work.

Moreover, Gucklhorn (1969) demonstrated that 400 mg/kg/day of DCP for 90 days did not cause toxic effect in rats.

During the efficacy studies on the murine model of AE, and in accordance to the Guide of Care and Use of Laboratory Animals (National Research Council, 2011), the appearance and behavior of mice were normal throughout the experimental period.

In the therapeutic efficacy study, ABZ and the NP-DCP caused a significant decrease in the weight of the metacestodes and in the viability of protoscoleces recovered from the treated mice. All treatments induced ultrastructural alterations on the germinal layer of the murine cysts. However, the extension of damage was greater after NP-DCP-treatment. The treatment with ABZ and the NP-DCP also caused marked ultrastructural changes on protoscoleces recovered from murine cysts. In contrast, DCP only induced slight changes like the loss of hooks.

In the chemoprophylactic efficacy study, both DCP formulations did not cause a significant decrease in the weight of the metacestodes recovered from the treated mice. In contrast, a significant reduction in cyst weight was observed after the administration of ABZ. However, the ultrastructural alterations observed in the germinal layer of the cysts recovered from the DCP and NP-DCP groups were consistent with the changes caused by ABZ. Moreover, the NP-DCP enhanced the efficacy of the drug alone. The damage extension in the germinal layer was greater after the treatment with the nanoparticles and a significant decrease in the viability of the protoscoleces recovered from cysts was observed.

Interestingly, the ultrastructural changes induced by both DCP formulations on cysts and protoscoleces during the *in vivo* studies were similar to those described for other drugs (Naguleswaran *et al*., 2006; Spicher *et al*., 2008; Albani *et al*., 2015).

Silica is abundantly distributed in nature, it has good compatibility and is accepted as generally recognized as safe (GRAS) by the FDA. Silica nanoparticles have been widely used in cosmetics and as food additives approved by the FDA (Halas, 2008). In addition, silica nanoparticles have potential as drug delivery vehicles for medical and veterinary treatments and as pesticides in the field of agriculture (Barik *et al*., 2012). This is due to their large and stable surface area and stability *in vivo*. Moreover, physical and chemical properties of the surface of a nanoparticle determine its capability to adsorb biomolecules and to disrupt cell membranes. The chemical composition of the surface will eventually control the pharmacological effect of a nanomaterial. Therefore, an increase in the hydrophobicity of the surface of silica nanoparticles after being modified with organic groups stimulates the adhesion of the nanoparticles to microorganisms (Arce et al., 2012).

The use of immobilized drugs on silica nanoparticles allows a greater absorption of poorly-water soluble drugs. This could generate an increase in the circulation times. Thus, the improvement in the *in vivo* efficacy of the NP-DCP could be explained by an increase in the systemic availability of DCP.

In conclusion, our results demonstrated that the NP-DCP had a stronger activity than the drug alone and their therapeutic efficacy was similar to ABZ suspension against the larval stage of *E. multilocularis*. The manufacture of NP-DCP is scalable, cost-effective and controllable. Therefore, DCP could be a potential repurposed anti-echinococcal drug and this nanotechnology strategy could greatly enhance its efficacy.

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Conflict of interest. None.

Ethical standards. Female CF-1 mice (body weight 25 g ± 5) were used. The animals were housed in a temperature-controlled (22 ± 1 ºC), light-cycle (12 h light/dark cycle) room. Food and water were given *ad libitum*. Animal procedures and management protocols were approved by the Institutional Animal Care and Use Committee (RD 148/15) of the Faculty of Exact and Natural Sciences, National University of Mar del Plata, Mar del Plata, Argentina and carried out in accordance with the revised form of The Guide for the Care and Use of Laboratory Animals (National Research Council US, 2011). Unnecessary animal suffering was avoided throughout the study.

References

Adams ARD and Seaton DR (1959) Treatment of *Taenia saginata* infection with dichlorophen. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **53**, 5.

Albani CM and Elissondo MC (2014) Efficacy of albendazole in combination with thymol against *Echinococcus multilocularis* protoscoleces and metacestodes. *Acta Tropica* **140**, 61-67.

Albani CM, Pensel PE, Elissondo N, Gambino G and Elissondo MC (2015) *In vivo* activity of albendazole in combination with thymol against *Echinococcus multilocularis*. *Veterinary Parasitology* **212**, 193-199.

Arce VB, Bertolotti SG, Oliveira FJ, Airoldi C, Arques A, Santos-Juanes L, Gonzalez MC, Cobos CJ, Allegretti PE and Mártire DO (2012) Triplet state of 4-methoxybenzyl alcohol chemisorbed on silica nanoparticles. *Photochemical & Photobiological Sciences* **11**, 1032-1040.

Ayres M, Ayres Jr M, Ayres DL and Santos AS (2007) *BioEstat 5.0 - Aplicações estatísticas nas áreas das ciências bio-médicas*, 5th ed. Imprensa Oficial do Estado do Pará, Brazil.

Baker NC, Ekins S, Williams AJ and Tropsha A (2018) A bibliometric review of drug repurposing. *Drug Discovery Today* **23**, 661-672.

Barik TK, Kamaraju R and Gowswami A (2012) Silica nanoparticle: a potential new insecticide for mosquito vector control. *Parasitology Research* **111**, 1075-1083.

Biagi FF, Gómez Orozco L and Robledo E (1959) Efficacy of dichlorophen against *Hymenolepis nana*. Reprinted from *Boletín Médico del Hospital Infantil* **16**, 113-116.

Daniel-Mwambete K, Torrado S, Cuesta-Bandera C, Ponce-Gordo F and Torrado JJ (2004) The effect of solubilization on the oral bioavailability of three benzimidazole carbamate drugs. *International Journal of Pharmaceutics* **272**, 29-36.

Deplazes P, Rinaldi L, Alvarez Rojas CA, Torgerson PR, Harandi MF, Romig T, Antolova D, Schurer JM, Lahmar S, Cringoli G, Magambo J, Thompson RCA and Jenkins EJ (2017) Global distribution of alveolar and cystic echinococcosis. In: Thompson RCA, Deplazes P and Lymbery AJ (Eds.). *Advances in Parasitology. Echinococcus and echinococcosis, Part A.* Academic Press **95**, 315-493.

El-Moslemany RM, Eissa MM, Ramadan AA, El-Khordagui LK and El-Azzouni MZ (2016) Miltefosine lipid nanocapsules: intersection of drug repurposing and nanotechnology for single dose oral treatment of pre-patent schistosomiasis mansoni. *Acta Tropica* **159**, 142-148.

Elissondo MC, Ceballos L, Dopchiz M, Andresiuk V, Alvarez L, Sánchez Bruni S, Lanusse C and Denegri G (2007) *In vitro* and *in vivo* effects of flubendazole on *Echinococcus granulosus* metacestodes. *Parasitology Research* **100**, 1003-1009.

Elissondo MC, Dopchiz M, Ceballos L, Alvarez L, Sánchez Bruni S, Lanusse C and Denegri G (2006) *In vitro* effects of flubendazole on *Echinococcus granulosus* protoscoleces. *Parasitology Research* **98**, 317-323.

Escalada JP, Arce VB, Porcal GV, Biasutti MA, Criado S, García NA and Mártire DO (2014) The effect of dichlorophen binding to silica nanoparticles on its photosensitized degradation in water. *Water Research* **50**, 229-236.

Fukui M, Kaneko C and Ogawa A (1960) Studies on equine tapeworms and their intermediate hosts. 2. Studies on removal effects of bithionol, bithionol acetate and dichlorophen for equine tapeworm, *Anoplocephala perfoliata*. *Japanese Journal of Parasitology* **9**, 217-223.

Fukui M (1960) Studies on *Momezia expansa* and its intermediate host. V. Removal of sheep, tapeworms, *M. expansa* and *M. benedeni*, with bithionol and dichlorophen. *Journal of the Japan Veterinary Medical Association* **13**, 294-297.

Gemmell MA (1958) The efficiency of dichlorophen (2, 2′- dihydroxy- 5, 5- dichlorodipenyl methane) against *Echinococcus granulosus* infestations in dogs. *Australian Veterinary Journal* **34**, 249-252.

Grinenko NV (1964) The action of anthelmintics and their compounds on cestodes *in vitro*. *Meditsinskaya Parazitologiya i Parazitarnye Bolezni* **33**, 87-92.

Grüner B, Kern P, Mayer B, Gräter T, Hillenbrand A, Barth TEF, Muche R, Henne-Bruns D, Kratzer W and Kern P (2017) Comprehensive diagnosis and treatment of alveolar echinococcosis: a single-center, long-term observational study of 312 patients in Germany. *GMS Infectious Diseases* **5**, 1-12.

Gucklhorn IR (1969) Antimicrobials in cosmetics. Part 2. *Manufacturing chemist and aerosol news* **40**, 38-40.

Halas NJ (2008) Nanoscience under glass: the versatile chemistry of silica nanostructures. *ACS Nano* **2**, 179-183.

Hemphill A, Stadelmann B, Rufener R, Spiliotis M, Boubaker G, Müller J, Müller N, Gorgas D and Gottstein B (2014) Treatment of echinococcosis: albendazole and mebendazole-what else? *Parasite* **21**.

Irache JM, Esparza I, Gamazo C, Agüeros M and Espuelas S (2011) Nanomedicine: novel approaches in human and veterinary therapeutics. *Veterinary Parasitology* **180**, 47-71.

Jackson FC (1956) The treatment of tapeworm infestation with dichlorophen. *South African Medical Journal* **30**, 853-854.

Kern P, Menezes da Silva A, Akhan O, Müllhaupt B, Vizcaychipi KA, Budke C and Vuitton DA (2017) The echinococcoses: diagnosis, clinical management and burden of disease. In: Thompson RCA, Deplazes P and Lymbery AJ (Eds.). *Advances in Parasitology. Echinococcus and echinococcosus, Part B.* Academic Press **96**, 259-369.

Küster T, Stadelmann B, Rufener R, Risch C, Müller J and Hemphill A (2015) Oral treatments of *Echinococcus multilocularis*-infected mice with the antimalarial drug mefloquine that potentially interacts with parasite ferritin and cystatin. *International Journal of Antimicrobial Agents* **46**, 546-551.

Llinàs MC and Sánchez-García D (2014) Nanopartículas de sílice: preparación y aplicaciones en biomedicina. *Afinidad* **71**, 20-31.

Lundström-Stadelmann B, Rufener R, Ritler D, Zurbriggen R and Hemphill A (2019) The importance of being parasiticidal… an update on drug development for the treatment of alveolar echinococcosis. *Food and Waterborne Parasitology*, **12**, e00040.

Maddison JE, Page SW and Church DB (2008) *Small animal clinical pharmacology.* Vol. 5. Elsevier Health Sciences.

Miller TA (1966) Anthelmintic activity of toluene and dichlorophen against various stages of *Ancylostoma caninum* in young dogs. *American Journal of Veterinary Research* **27**, 1755-1758.

Naguleswaran A, Spicher M, Vonlaufen N, Ortega-Mora LM, Torgerson P, Gottstein B and Hemphill A (2006) *In vitro* metacestodicidal activities of genistein and other isoflavones against *Echinococcus multilocularis* and *Echinococcus granulosus*. *Antimicrobial Agents and Chemotherapy* **50**, 3770-3778.

National Research Council US (2011) *Guide for the Care and Use of Laboratory Animals*, 8th ed. National Academies Press, US, Washington DC.

Panic G, Duthaler U, Speich B and Keiser J (2014) Repurposing drugs for the treatment and control of helminth infections. *International Journal for Parasitology: Drugs and Drug Resistance* **4**, 185-200.

Rapson EB, Jenkins DC and Topley P (1985) *Trichostrongylus colubriformis*: *in vitro* culture of parasitic stages and their use for the evaluation of anthelmintics. *Research in Veterinary Science* **39**, 90-94.

Reuter S, Buck A, Grebe O, Nüssle-Kügele K, Kern P and Manfras BJ (2003) Salvage treatment with amphotericin B in progressive human alveolar echinococcosis. *Antimicrobial Agents and Chemotherapy* **47**, 3586-3591.

Rufener R, Ritler D, Zielinski J, Dick L, da Silva ET, da Silva Araujo A, Joekel DE, Czock D, Goepfert C, Moraes AM and de Souza MVN (2018) Activity of mefloquine and mefloquine derivatives against *Echinococcus multilocularis*. *International Journal for Parasitology: Drugs and Drug Resistance* **8**, 331-340.

Sakamoto T (1973) Studies on echinococcosis XXV: anthelmintic action of drugs on larval *Echinococcus multilocularis in vitro*. *Japanese Journal of Veterinary Research* **21**, 73-91.

Sen AB and Hawking F (1960) Screening of cesticidal compounds on a tapeworm *Hymenolepis nana in vitro*. *British Journal of Pharmacology and Chemotherapy* **15**, 436-439.

Siles-Lucas M, Casulli A, Cirilli R and Carmena D (2018) Progress in the pharmacological treatment of human cystic and alveolar echinococcosis: Compounds and therapeutic targets. *PLOS Neglected Tropical Diseases* **12**, e0006422.

Spicher M, Roethlisberger C, Lany C, Stadelmann B, Keiser J, Ortega-Mora LM, Gottstein B and Hemphill A (2008) *In vitro* and *in vivo* treatments of *Echinococcus* protoscoleces and metacestodes with artemisinin and artemisinin derivatives. *Antimicrobial Agents and Chemotherapy* **52**, 3447-3450.

Spiliotis M and Brehm K (2009) Axenic i*n vitro* cultivation of *Echinococcus multilocularis* metacestode vesicles and the generation of primary cell cultures. In: Rupp S and Sohn K (eds). *Host-pathogen interactions. Methods in Molecular Biology, vol 470.* Humana Press, pp. 245-262.

Stettler M, Rossignol JF, Fink R, Walker M, Gottstein B, Merli M, Theurillat R, Thormann W, Dricot E, Segers R and Hemphill A (2004) Secondary and primary murine alveolar echinococcosis: combined albendazole/nitazoxanide chemotherapy exhibits profound anti-parasitic activity. *International Journal for Parasitology* **34**, 615-624.

Tappe D, Müller A, Frosch M and Stich A (2009) Limitations of amphotericin B and nitazoxanide in the treatment of alveolar echinococcosis. *Annals of Tropical Medicine and Parasitology* **103**, 177-181.

Thompson DP and Geary T (1995) The structure and function of helminth surfaces. In Harr J and Muller M (eds). *Biochemistry and molecular biology of parasites.* Academic, London, UK, pp. 203-232.

Vico TA, Arce VB, Fangio MF, Gende LB, Bertran CA, Mártire DO and Churio MS (2016) Two choices for the functionalization of silica nanoparticles with gallic acid: characterization of the nanomaterials and their antimicrobial activity against *Paenibacillus larvae*. *Journal of Nanoparticle Research* **18**, 348.

Yamarik TA (2004) Safety assessment of dichlorophene and chlorophene. *International Journal of Toxicology* **23**, 1-27.

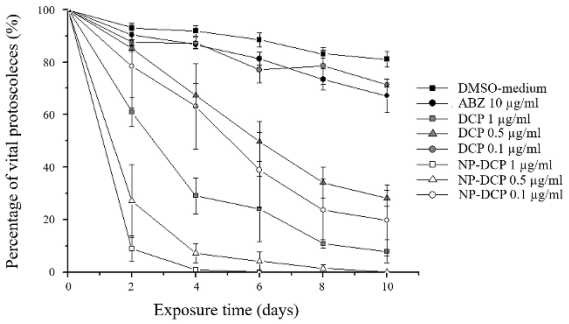

Fig. 1. Survival of *E. multilocularis* protoscoleces after *in vitro* exposure to different concentrations of DCP and NP-DCP. Each point represents the mean percentage ± SD of vital protoscoleces from three different experiments.

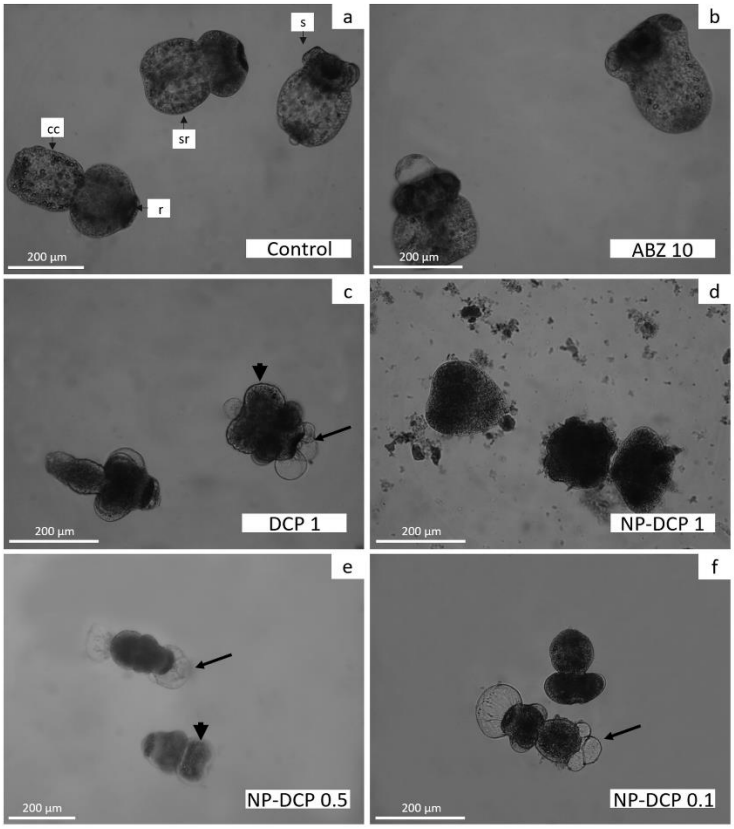

Fig. 2. Light microscopy of *E. multilocularis* protoscoleces incubated *in vitro* with ABZ, DCP, and NP-DCP. a- Control evaginated protoscoleces, 10 days p.i. (s sucker, sr soma region, r rostellar region, cc calcareous corpuscles). b- Protoscoleces treated with 10 µg/ml of ABZ during 10 days. c- Protoscoleces incubated for 2 days with 1 µg/ml of DCP. Observe the formation of blebs in the tegument (arrow) and the contraction of the soma region (head arrow). d- Dead protoscoleces, 2 days p.i. with 1 µg/ml of NP-DCP. Observe the loss of the characteristic morphology of the larvae. e- Protoscoleces with the presence of blebs in the tegument (arrow) and contraction of the soma region (head arrow, 0.5 µg/ml of NP-DCP, 2 days p.i.). f- Protoscoleces incubated with NP-DCP (4 days, 0.1 µg/ml). Note the presence of blebs (arrow).

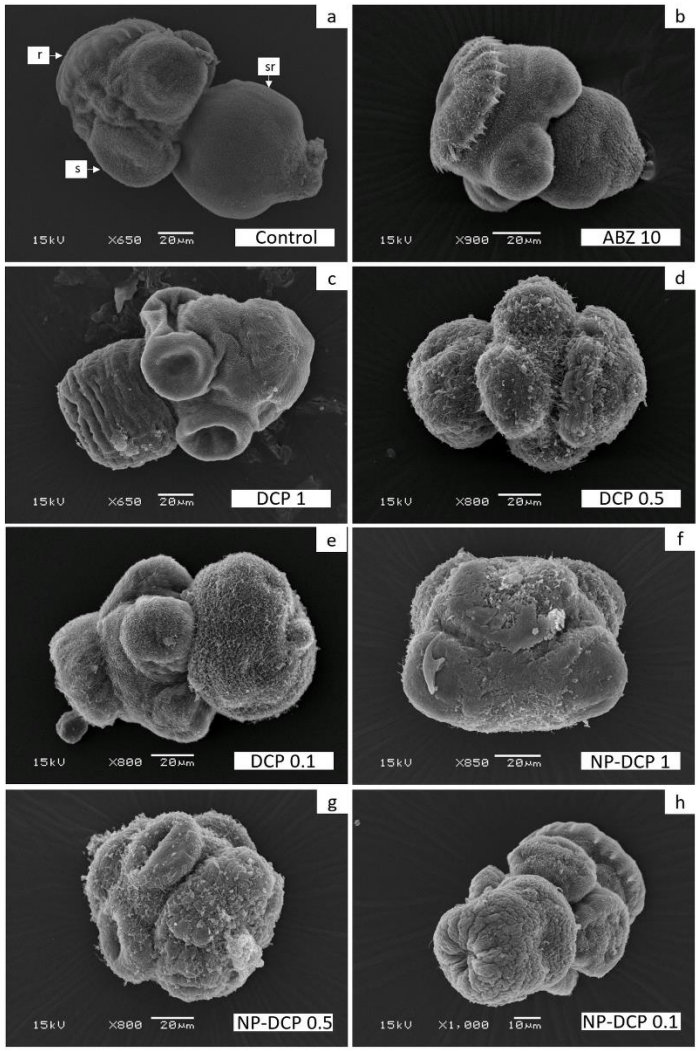

Fig. 3. Scanning electron microscopy of *E. multilocularis* protoscoleces incubated *in vitro* with ABZ, DCP, and NP-DCP. a- Evaginated control protoscolex (6 days p.i.). Observe the intact microtriches (r rostello region with hooks, s sucker, sr soma region). b- Protoscolex incubated with 10 µg/ml of ABZ for 6 days, with contraction of the soma and alteration of tegument in that region. c- Protoscolex treated with 1 µg/ml of DCP for 2 days, with an intact scolex region. The soma is contracted and the tegument altered. d- Protoscolex treated 6 days with 0.5 µg/ml of DCP. Observe the loss of microtriches and hooks, the contraction of the soma, and the presence of blebs in the

tegument. e- Protoscolex with contraction of soma region and tegumental alterations (6 days p.i., 0.1 μg/ml of DCP). The scolex region remains unchanged. f- Protoscolex showing a total loss of morphology and blebs in the tegument (1 μg/ml of NP-DCP, 2 days p.i.). g- Protoscolex incubated with 0.5 μg/ml of NP-DCP (2 days p.i.). Observe the total loss of microtriches, the presence of blebs in the tegument, and the contracted soma region. h- Protoscolex with an intact scolex region, but with alterations on the tegument of the soma region (0.1 μg/ml of NP-DCP, 2 days p.i.).

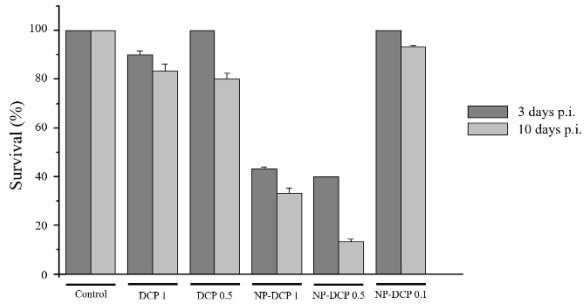

Fig. 4. Survival of *E. multilocularis* vesicles after 3 and 10 days of *in vitro* exposure to different concentrations of DCP and NP-DCP. The criteria for metacestode vesicles viability was the collapse of the germinal layer. Each bar represents the mean percentage ± SD of vital vesicles.

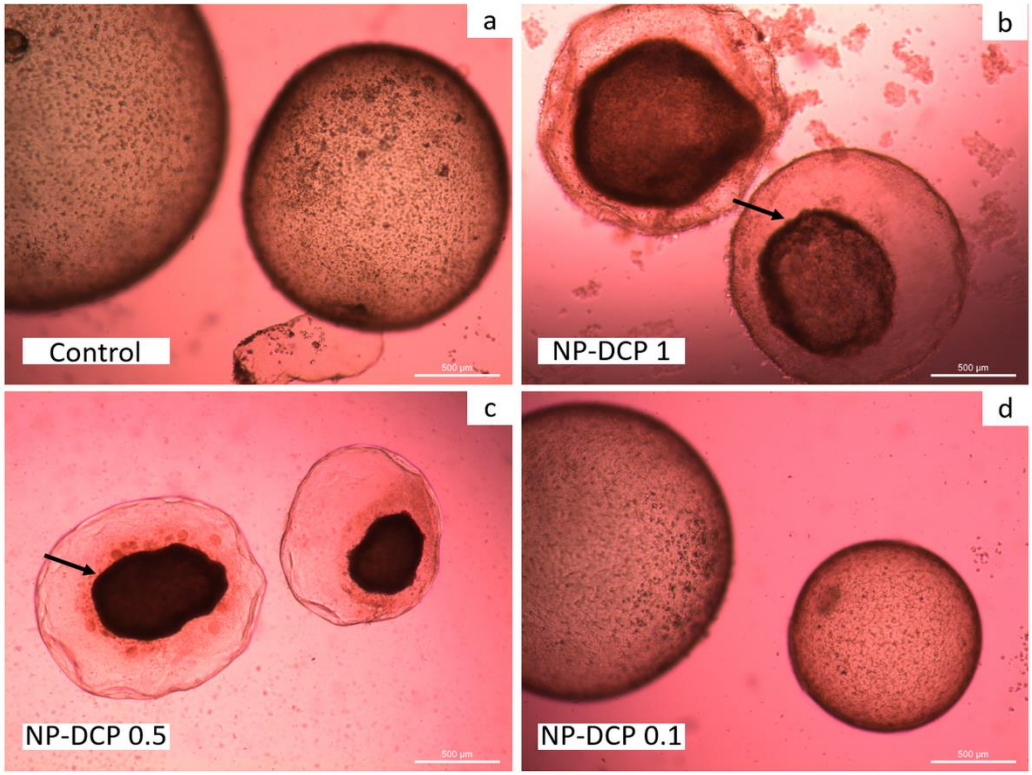

Fig. 5. *In vitro* treatment of metacestode vesicles of *E. multilocularis* with different concentrations of NP-DCP. a- Control metacestodes after 10 days of incubation with an intact germinal layer. b- Metacestode vesicles incubated with 1 µg/ml of NP-DCP for 1 day. Note the rapid collapse of the germinal layer (arrow). c- Vesicles with germinal layer detached from the laminar layer (arrow) after 2 days of incubation with 0.5 µg/ml of NP-DCP. d- Metacestodes incubated for 10 days with 0.1 µg/ml of NP-DCP.

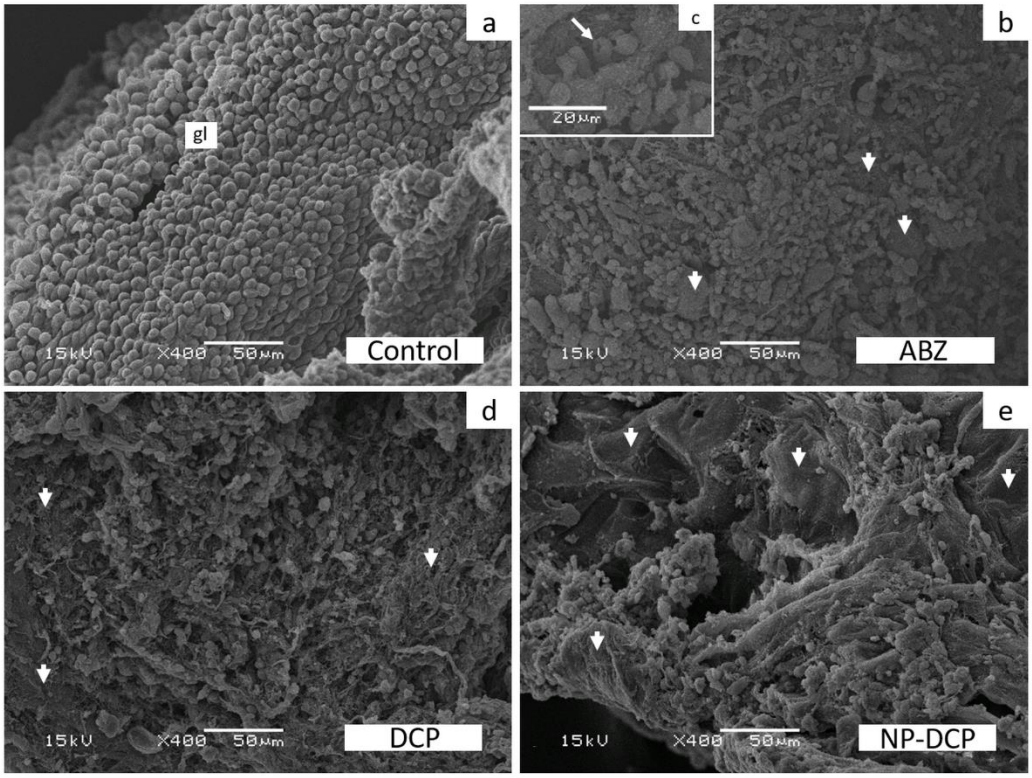

Fig. 6. Scanning electron microscopy of *E. multilocularis* cysts recovered from infected mice treated with 25 mg/kg of ABZ, 4 mg/kg of DCP or 4 mg/kg of NP-DCP, belonging to the therapeutic efficacy study. a- Control cyst with an intact germinal layer (gl). b- Cyst recovered from mice treated with ABZ. Observe the altered germinal layer, with loss (arrowhead) and alteration of cells. c- High magnification image showing altered cells (arrow). d- Cyst obtained from treatment with DCP. Areas with loss of cells (arrowhead) are observed as well as altered cells. e- Metacestode from NP-DCP treated group, with extensive areas of the germinal layer without cells (arrowhead). Altered cells and cellular debris can be observed.

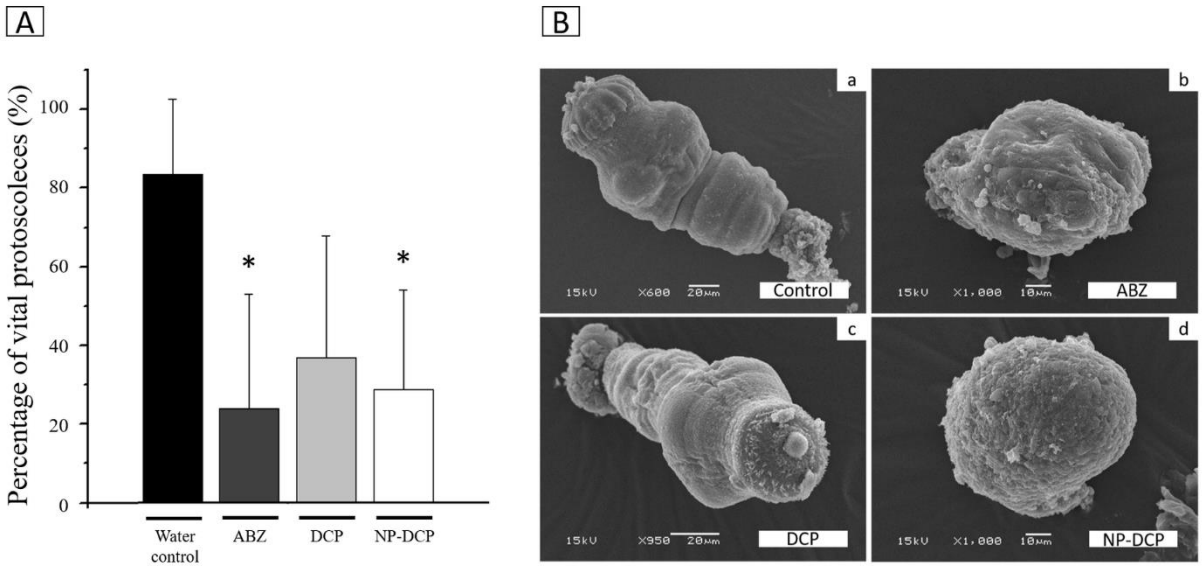

Fig. 7. (A) Viability and (B) scanning electron microscopy of *E. multilocularis* protoscoleces obtained from cysts recovered from mice belonging to control and treated groups of the therapeutic efficacy study. (A) Statistically significant differences were found with the water control group: * ($P < 0.05$). (B) a- Evaginated control protoscolex. b- Protoscolex with total loss of morphology, obtained from mice treated with 25 mg/kg of ABZ. c- Protoscolex recovered from mice treated with 4 mg/kg of DCP. d- Protoscolex completely altered, obtained from mice treated with 4 mg/kg of NP-DCP.

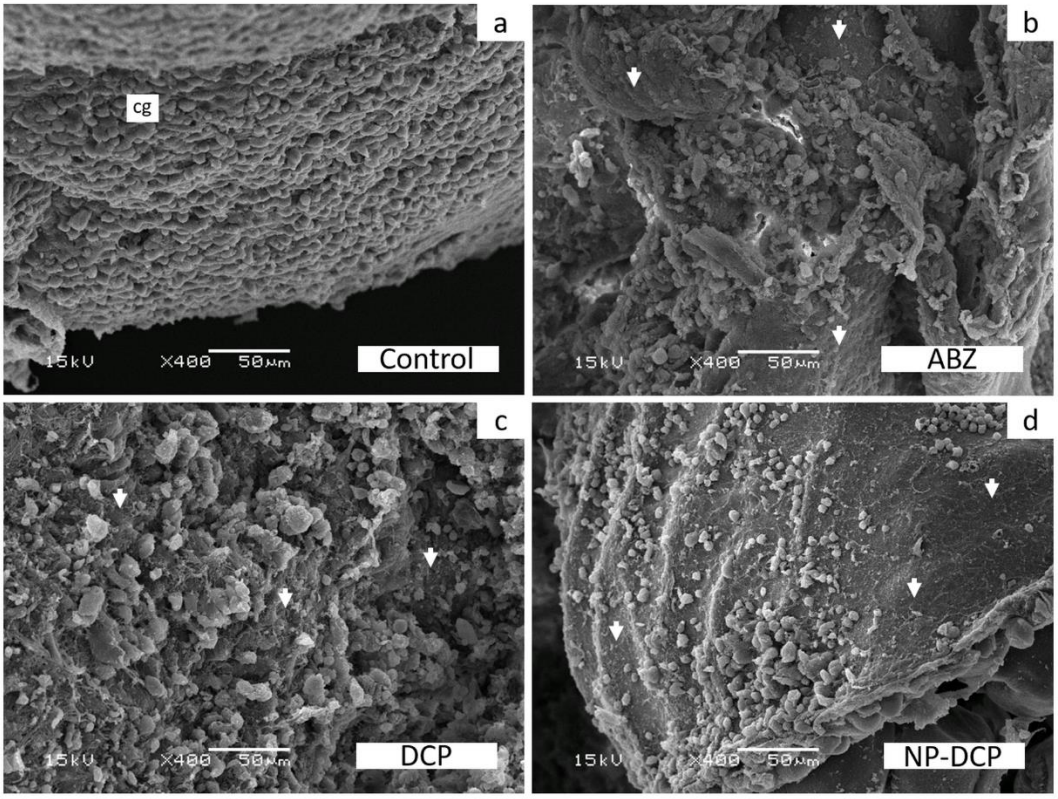

Fig. 8. Scanning electron microscopy of *E. multilocularis* cysts recovered from infected mice treated with 25 mg/kg of ABZ, 4 mg/kg of DCP or 4 mg/kg of NP-DCP, belonging to the chemoprophylactic efficacy study. a- Metacestode recovered from the mice of the control group showing the characteristic ultrastructure of the germinal layer (gl). b- Cysts from ABZ treated mice. Observe the large areas of the germinal layer without cells (arrowhead). c- Metacestode of mice belonging to DCP group. Observe the areas without cells in the germinal layer (arrowhead). d- Metacestode obtained from mice of NP-DCP group. Note the extensive areas of the germinal layer with loss of cells (arrowhead).

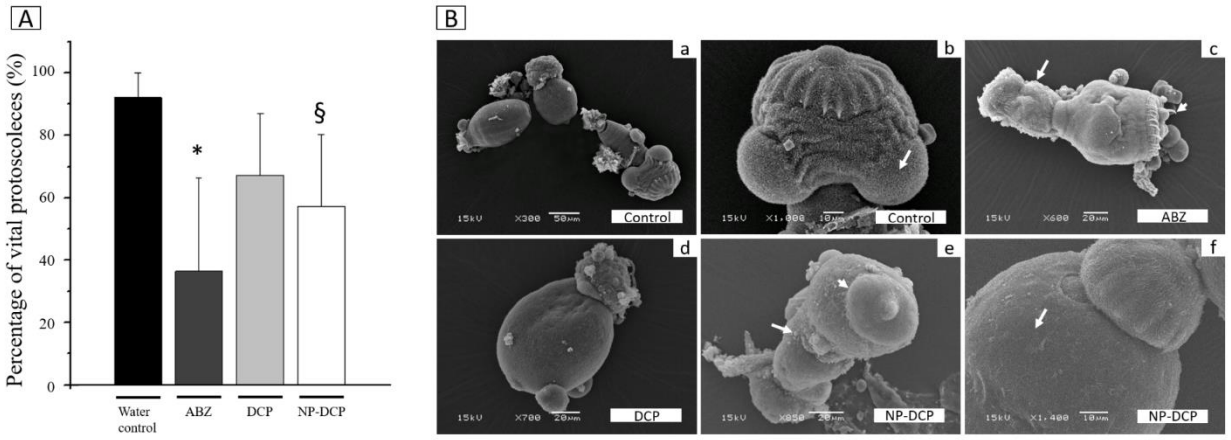

Fig 9. (A) Viability and (B) scanning electron microscopy of *E. multilocularis* protoscoleces isolated from cysts obtained of treated mice during the chemoprophylactic efficacy study. (A) Statistically significant differences were found with the water control group: * ($P < 0.001$) and § ($P < 0.01$). (B) a- Invaginated and evaginated control protoscoleces. b- Detail of the scolex of an evaginated control protoscolex. Observe the microtriches (arrow). c- Protoscolex obtained from the treatment with 25 mg/kg of ABZ. Observe the rostellar disorganization (arrowhead) and the contraction of the soma (arrow). d- Invaginated protoscolex recovered from treatment with 4 mg/kg of DCP. e- Protoscolex isolated from cysts of the NP-DCP group. Note the loss of hooks (arrowhead) and microtriches and the presence of vesicles in the tegument (arrow). f- Evaginated protoscolex obtained from mice treated with 4 mg/kg of NP-DCP. Observe the lack of microtriches on the scolex region (arrow).

Table 1. Therapeutic and chemoprophylactic efficacy studies. Mean weights (g ± SD) of cysts obtained (10 weeks post-infection) from *E. multilocularis* infected mice. Treatments were performed for 30 days every 24 hours.

	Therapeutic efficacy	**Chemoprophylactic efficacy**
	Mean weight of cysts (g ± SD)	
Water control	4.48 ± 2.3	4.57 ± 1.9
NPs control	4.64 ± 2.1	3.77 ± 2.7
ABZ	1.71 ± 0.8[a,b]	1.94 ± 0.4[c]
DCP	2.52 ± 1.1	3.31 ± 1.7
NP-DCP	2.01 ± 0.7[a,b]	3.63 ± 1.8

In therapeutic study, statistically significant differences were found between:

[a] The water control group and the following treatment: ABZ group ($P < 0.01$) and NP-DCP group ($P < 0.05$).

[b] The NPs control group and the following treatment: ABZ group and NP-DCP group ($P < 0.01$).

In chemoprophylactic study, statistically significant differences were found with:

[c] The water control group ($P < 0.05$).