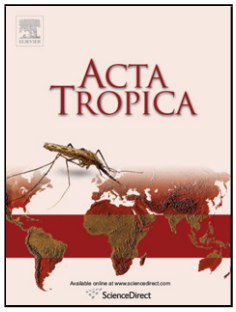


Title: Elucidating the influence of praziquantel nanosuspensions on the *in vivo* metabolism of *Taenia crassiceps* cysticerci.

Author: Luciana Damacena Silva Eva Carolina Arrúa Dayanne Amaral Pereira Carolina Miguel Fraga Tatiane Luiza da Costa Andrew Hemphill Claudio Javier Salomon Marina Clare Vinaud

PII: S0001-706X(16)30359-X
DOI: http://dx.doi.org/doi:10.1016/j.actatropica.2016.06.002
Reference: ACTROP 3948

To appear in: *Acta Tropica*

Received date: 9-2-2016
Revised date: 24-5-2016
Accepted date: 3-6-2016

Please cite this article as: Silva, Luciana Damacena, Arrúa, Eva Carolina, Pereira, Dayanne Amaral, Fraga, Carolina Miguel, Costa, Tatiane Luiza da, Hemphill, Andrew, Salomon, Claudio Javier, Vinaud, Marina Clare, Elucidating the influence of praziquantel nanosuspensions on the in vivo metabolism of Taenia crassiceps cysticerci.Acta Tropica http://dx.doi.org/10.1016/j.actatropica.2016.06.002

Highlights

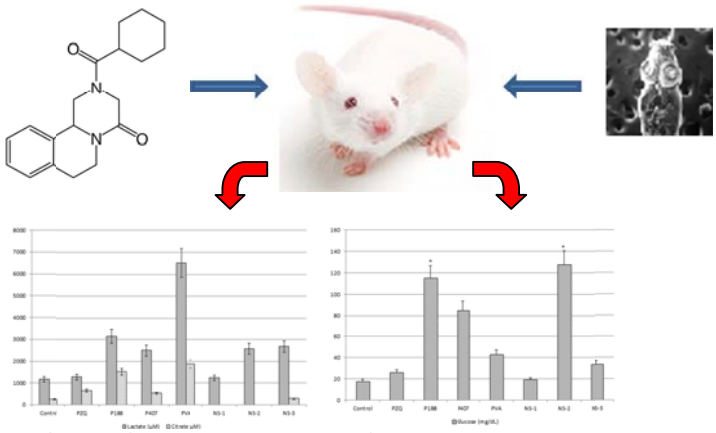
The effect of the nanosuspensions on the energetic metabolism of cysticerci was proved.

PZQ nanosuspensions increased the glycolysis in *T. crassiceps* cysticerci.

Remarkable metabolic alterations produced by the stabilizers of the nanosuspensions were observed.

Praziquantel nanosuspensions were succesfully prepared by nanoprecipitation process.

Graphical abstract



Modification of the energetic metabolism of cysticerci *in vivo* by PZQ nanosuspensions

Elucidating the influence of praziquantel nanosuspensions on the *in vivo* metabolism of *Taenia crassiceps* cysticerci.

LUCIANA DAMACENA SILVA,[1] EVA CAROLINA ARRÚA,[2] DAYANNE AMARAL PEREIRA,[1] CAROLINA MIGUEL FRAGA,[3] TATIANE LUIZA DA COSTA,[3] ANDREW HEMPHILL,[4] CLAUDIO JAVIER SALOMON,[2,5*] and MARINA CLARE VINAUD[3*]

[1]State University of Goias, Br 153, Km 99, Anápolis-Goiás, Brazil. [2]Institute of Chemistry, IQUIR-CONICET. National Council Research. Suipacha 531, 2000, Rosario, Argentina. [3]Tropical Pathology and Public Health Institute, 235 street, Federal University of Goias, Goiania, Brazil, CEP: 74605-050. [4]Institute of Parasitology, Vetsuisse Faculty, University of Berne Länggass-Strasse 122, CH-3012 Berne, Switzerland. [5]Pharmacy Department, Faculty of Pharmaceutical and Biochemical Sciences, National University of Rosario, Suipacha 531, 2000, Rosario, Argentina.

* Corresponding authors:

Marina C. Vinaud. Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, rua 235, s/n, Setor Universitário, Goiânia, Goiás, CEP 74650-050, Brazil; email address: marinavinaud@gmail.com.

Claudio J. Salomon. Pharmacy Department, Faculty of Pharmaceutical and Biochemical Sciences, National University of Rosario, Suipacha 531, 2000, Rosario, Argentina; email address: csalomon@fbioyf.unr.edu.ar

SUMMARY

The aim of this work was to develop nanosuspensions of praziquantel (PZQ) and to evaluate their influence on the energetic metabolism of cysticerci inoculated in BALB/c mice. We analyzed metabolic alterations of glycolytic pathways and the tricarboxylic acid cycle in the parasite. The nanosuspensions were prepared by precipitation and polyvinyl alcohol (PVA), poloxamer 188 (P188) and poloxamer 407 (P407) were used as stabilizers. Nanosuspension prepared with PVA had a particle size of 100 nm, while P188- and P407-based nanosuspensions had particle sizes of 74 nm and 285 nm, respectively. The zeta potential was -8.1, -8.6, and -13.2 for the formulations stabilized with PVA, P188 and P407, respectively. Treatments of *T. crassiceps* cysticerci-infected mice resulted in an increase in glycolysis organic acids, and enhanced the partial reversion of the tricarboxylic acid cycle, the urea cycle and the production of ketonic bodies in the parasites when compared to the groups treated with conventional PZQ. These data suggest that PZQ nanosuspensions greatly modified the energetic metabolism of cysticerci *in vivo*. Moreover, the remarkable metabolic alterations produced by the stabilizers indicate that further studies on nanoformulations are required to find potentially suitable nanomedicines.

Keywords: Nanosuspensions; Praziquantel; energetic metabolism; *Taenia crassiceps; in vivo*

INTRODUCTION

Cestode parasites belonging to the family Taeniidae, including the genera *Echinococcus* and *Taenia* inflict of serious diseases such as alveolar and cystic echinococcosis, caused by the larval (metacestode) *E. multilocularis* and *E. granulosus*), taeniosis (caused by the adult stage of *T. solium* and *T. saginata*) and cysticercosis (caused by the larval stage (cysticercus) of *T. solium*). *Taenia crassiceps* is another family member, which primarily infect rodents as intermediate hosts and canids as final hosts. *T. crassiceps* rarely infects humans, but parasites can enter their host by ingestion of contaminated food and water. In immunecompetent individuals, *T. crassiceps* is mostly eliminated before permanent damage is done, however, in other cases parasite larvae invade the vitreous and retina of the eye, and can cause blindness. It is also important to mention that *T. crassiceps* is described as an opportunistic infection in immunocompromised patients (Ntoukas et al. 2013). Praziquantel (PZQ) is the drug of choice to treat helminth tissue infections such as schistosomiasis and cysticercosis which are both tropical neglected diseases (Del Brutto 2014; Secor 2015). PZQ also exerts a remarkable efficacy on the adult stages of the Taeniidae, and on cultured or inoculated cysticerci (Palomares et al. 2004; Chai et al. 2013; Cioli et al. 2014). However, its mode of action on the main metabolic pathways used by these parasites has been only scarcely studied (Vinaud et al. 2007; Vinaud et al. 2008). It has been determined that *in vitro* treatment of *T. crassiceps* cysticerci with PZQ lead to inhibition of the glucose uptake which resulted in decrease of lactate concentrations in the culture medium and a preference for aerobic metabolism in such parasites (Vinaud et al. 2008). PZQ is a heterocyclic pyrazinoisoquinoline, and as other structurally similar molecules it is poorly soluble in water (0.4 mg/mL), which can lead to variable oral bioavailability and therapeutic failures. In this regard, the development of

nanomedicines has been widely investigated as an attractive alternative to improve the *in vivo* performance of hydrophobic biologically active molecules (Merisko-Liversidge et al. 2011). Particularly, submicron formulations of PZQ were developed including polymeric nanoparticles and solid lipid nanoparticles (Mainardes et al. 2006; Xie et al. 2010). However, to date, the formulation of PZQ nanosuspensions has not been described. Although these nanosystems exhibit several advantages including their simplicity in preparation and general applicability, the reduction of particle size may produce uncontrolled aggregation of nanoparticles due to inter-particle forces (Muller et al. 2001). To avoid this, stabilizers such as poloxamers, sodium lauryl sulphate, polisorbates, poly(vinyl) alcohol and polyvinylpirrolidones are added to the nanosuspensions (Singh, 2010). These macromolecules are used in the design of pharmaceutical formulations and are believed to be pharmacologically inert (Rowe et al. 2009). Among them, non-ionic stabilizers are widely applied in the development of nanoformulations due to their hydrophobic properties, their capacity to increase the aqueous solubility of lipophilic drugs and their effect on enhancing the oral bioavailability of hydrophobic molecules (Budijono et al., 2010). In this study, three non-ionic stabilizers were selected: the Poloxamers 188 (P188) and 407 (P407) and Poly(vinyl alcohol) (PVA). Poloxamers are non-ionic poly (ethylene oxide) (PEO)-poly (propylene oxide) (PPO) copolymers with interesting properties as surfactants and solubilizing molecules in pharmaceutical formulations, while PVA is a well-established excipient used in various biomedical and pharmaceutical products due to its non-toxic, non-carcinogenic, and bioadhesive properties. PVA also is known as a good stabilizer of submicron particles. These molecules were selected to decrease the chances of Ostwald ripening and to improve the stability of the suspension by providing a steric barrier (Junghanns et al. 2008).

The aim of this work was to evaluate the influence of PZQ nanosuspensions formulated with P188 (NS-1), P407 (NS-2) and PVA (NS-3) on the *in vivo* metabolism of cestode larval stages. *Taenia crassiceps* cysticerci were employed as experimental model. Also, these additives were evaluated, for the first time, without PZQ in order to determine any changes in glycolysis, the tricarboxylic acid (TCA) cycle and fatty acid metabolism. To our knowledge, this is the first study on the effects of such nanosuspensions and their additives on the metabolic pathways in resident parasites.

MATERIALS AND METHODS

Materials

Praziquantel was donated by Laboratorio Proagro (Rosario, Argentina). Lutrol® F-68 (P188) and Lutrol® F127 (P407) were donated by BASF SE (Ludwigshafen, Germany). Polyvinyl alcohol (PVA) (MW 13,000 to 23,000, 87-89% hydrolysis degree) was supplied by Sigma-Aldrich Chemicals (St. Louis, MO, USA). Purified water (Milli-Q, Millipore Corporation, Billerica, MA) was used. The reagents and chemicals used for analytical purpose were of chromatography grade.

Preparation of nanosuspensions

PZQ nanosuspensions were prepared by the solvent diffusion method. PZQ (800 mg) was dissolved in 20 ml of ethanol. The resulting solution was injected (1 ml min^{-1}) into 50 ml of water containing 250 mg (0.5% w/v) of the chosen stabilizer under magnetic stirring (500 rpm). The resulting emulsion was then stirred (500 rpm) for 18 h at room temperature to allow solvent evaporation and nanoparticle formation. Nanoparticles were recovered by centrifugation using a MIKRO 220 Hettich centrifuge (Andreas

Hettich GmbH & Co.KG, Tuttlingen, Germany) for 20 min (15000 rpm) and washed twice with distilled water to remove free drug. The obtained PZQ nanosuspensions were dispensed into 25 mL beakers and frozen overnight at -20 ^{o}C and freeze-dried (48 hours).

Particle Size and Zeta Potential Measurement

The particle size of the PZQ nanosuspensions was determined by photon correlation spectroscopy. The samples were prepared by tenfold dilution of 1ml of the nanosuspension with distilled water. The zeta potential was determined by the electrophoretic mobility of nanoparticles at 25°C using the SZ-100 Horiba instrument (HORIBA Instruments Inc. California, USA). All the determinations were carried out in triplicate.

Maintenance of the T. crassiceps and treatment of infected mice.

The biological cycle of *T. crassiceps* (ORF strain) has been maintained in the animal facilities of the Tropical Pathology and Public Health Institute, Federal University of Goias, (IPTSP/UFG) by serial passages of cysticerci since 2002. For infection, 10 initial phase cysticerci were inoculated into the peritoneal cavity of 8 to 12 week old female BALB/c mice where they multiplied by budding. 90 days post-infection, the animals were euthanized and necropsied, and recovered cysticerci used for further infections. (Espíndola et al. 2002; Vinaud et al. 2008, Fraga et al. 2012). The mice received daily care, acidified water and standard rations. The ethical principles for animal experimentation professed by the Brazilian society of laboratory animal sciences (Sociedade Brasileira de Ciência em Animais de Laboratório/SBCAL) and the Legislation for the protection of animals used for scientific purposes (Directive

2010/63/EU) were followed and this study was authorized by the Committee for Ethical Research of the Federal University of Goias (CoEp/UFG) (registration number 037/15).

For treatment experiments, mice infected with 10 *T. crassiceps* cysticerci (Vinaud et al. 2008) were gavage treated with a single dose of the formulations at day 30 post-infection. 24 hours post-treatment they were euthanized, and the recovered cysticerci were assessed for biochemical effects of the formulations. The infected mice were divided into the following groups of 5 animals each, and received the following treatments: negative control (no treatment); positive control (treatment with 50mg/kg of PZQ); P188 (50mg/kg of P188); P407 (50mg/kg P407); PVA (50mg/kg of PVA); NS-1 (50mg/kg of PZQ:P-188 nanosuspension); NS-2 (50mg/kg of PZQ:P-407 nanosuspension); NS-3 (50mg/kg of PZQ:PVA nanosuspension).

Biochemical analysis of cysticerci

The cysticerci were recovered from the mice and were frozen in liquid nitrogen, and then homogenized in Tris-HCl 0.5M buffer, pH 7.6, with a protease inhibitor (SigmaFast Protease inhibitor cocktail, Sigma). The obtained extract was centrifuged at 15,652 x g (10000 rpm/10 min/4ºC). 500 µl of the supernatant was processed by a solid phase extraction column for the organic acids extraction and subsequent HPLC analysis (Vinaud et al. 2009, Fraga et al. 2012). Another 500 µl of the supernatant was analyzed by spectrophotometry (Architec C8000 Plus) in order to quantify glucose, urea and creatinine content. The organic acids were identified through HPLC, as previously detailed (Vinaud et al. 2009). The organic acids used as glycolysis indicators were pyruvate and lactate, while markers for the TCA cycle were citrate, alfa-ketoglutarate, succinate, fumarate, malate and oxaloacetate. Markers of the fatty acid metabolism include acetate, acetoacetate, beta-hydroxybutirate and propionate (Vinaud et al. 2009).

Statistical Analysis

The statistical analysis was performed using the Sigma Stat 2.3 software. Descriptive statistics were applied to determine the mean and standard deviation and to evaluate the differences between the groups analyzed. The variables were tested for normal distribution and homogeneous variance. Analysis of variance was used followed by Holm-Sidak posttest. The differences were considered significant when $p<0.05$.

RESULTS

Particle size and zeta potential characterization of PZQ nanosuspensions

The mean particle size of the nanosuspensions prepared with P188 (NS-1) and P407 (NS-2) were 74.6 nm and 282 nm, respectively while preparation with PVA (NS-3) resulted in a mean particle size of 105.4 nm. The zeta potential of nanoparticles prepared with P188 (NS-1), P407 (NS-2) and PVA (NS-3) were -8.1, -8.6, and -13.2, while the polydispersity indeces (PI) were 0.19, 0.30 and 0.24, respectively.

Effects on glycolysis.

As seen in Figure 1, the glucose concentration was increased in the group treated with P188 alone and in the NS-2 group ($p<0.05$). A 100-fold increase in pyruvate production was detected in the NS-1 and NS-2 treated groups ($p<0.05$) (Table 1). Lactate production by the PVA treated group was significantly higher than in the control group, conventional PZQ and the NS-3 treated group ($p<0.05$). In spite of the lactate production being slightly increased in the other treated groups, no significant differences were observed.

Insert Table 1

Insert Figure 1

Effects on the tricarboxilic acid (TCA) cycle

Negative control cysticerci presented a complete TCA cycle detected by the presence of alfa-ketoglutarate. All the treatments induced the fumarate reductase partial reverse of the TCA cycle. The fumarate reductase pathway is also known as the malate dismutation pathway in which the phosphoenolpyruvate produced from glycolysis is converted into cytoplasmic malate. This malate is transported into the mitochondria and is degraded in a split pathway. One portion of this organic acid is oxidized into acetate and another portion is reduced to fumarate and succinate, which can be excreted, or metabolized into propionate, as already described (Tielens and Van Hellemond 2005). Citrate and malate production was undetectable in the NS-1 and NS-2 treated groups. However in the P188, P407, PVA and NS-3 treated groups the production of these two organic acids was increased when compared to the control group and the group treated with conventional PZQ ($p<0.05$) (Table 2). Oxaloacetate production was increased in all treated groups ($p<0.05$) and, interestingly, the NS-2 group exhibited increased production of this organic acid, with a 32-fold increase compared to the control group and 3-fold increase compared to the PZQ treated group. Succinate production was not detected in the PVA and NS-3 treated groups, but was increased ($p<0.05$) in the other treatment groups. Fumarate and malate production, which are precursors to the succinate production via the fumarate reductase pathway, were significantly reduced in the NS-3 treated group ($p<0.05$) compared to the other treatment groups.

Insert Table 2

Insert Figure 2

Effects on the production of ketonic bodies and the urea cycle

Acetate production was significantly increased in the P188 and PVA treated groups when compared to the control group and PZQ treated group ($p<0.05$), but was not detected in any other treatment group (Table 3). Acetoacetate and beta-hydroxybutirate production was increased in all PZQ-nanosuspension (NS-1, NS-2, NS-3) treated groups ($p<0.05$) when compared to the control group, the group treated with conventional PZQ and the three groups treated with polymers alone. In addition, urea was increased in the P188 and P407 treated groups and, also, in the NS-2 treated group when compared to the control and PZQ treated group ($p<0.05$).

Insert Table 3

DISCUSSION

PZQ is one of the most frequently prescribed agents to treat several parasitic infections. The drug is generally well tolerated, has a broad-spectrum activity, but it is of utmost importance to improve its efficacy [1,3]. In this study, the nanoprecipitation technique, also known as bottom-up process, was successfully applied to produce PZQ nanoparticles, and P188, P407 and PVA polymers were used as stabilizers at 0.5% w/v. Even though nanoprecipitation is a very useful approach to solve the issues associated with poor solubility and low bioavailability of lipophilic compounds, the high surface energy of nano-sized particles may lead to an agglomeration of nanocrystals. As already described, polymers and macromolecules are able to stabilize such nanodispersions avoiding such agglomeration by providing steric or ionic barriers. As reported, P-188 and 407, as well as PVA, are well known efficient steric stabilizer applied to the formulation of nanoparticles and nanocrystals (Müller, 1991). In this case, P-188 gave

the smallest PZQ nanocrystals with a narrow size distribution, while both the size and PDI values were increased when P-407 and PVA were used. These findings confirm the postulate that the grade and/or type of polymers used as stabilizers may have a remarkable influence on the size of the nanoparticles (Reich, 1997; Lee et al., 2008; Dong et al., 2015). Another factor determining the physical stability of nanosuspensions is the particle charge. In this case, both poloxamers and PVA contributed to the formulation stability by means of steric effects. However, it should be noted that adsorption of a steric stabilizer layer may lead to a reduction of the zeta potential value. Although the zeta potential of the PZQ nanosuspensions was found to be between 8 and 13mV, it may be enough to stabilize the system in combination with steric stabilization produced by the polymers (Müller, 1991). Even though PZQ nanosuspensions would be very useful as a novel anti-parasitic dosage form, the biochemical analysis of their antihelminthics mode of action is important in order to understand whether such improvements may present an increased effect on the metabolic pathways of the parasite.

The increase in glucose concentrations in *T. crassiceps* cysticerci treated with PZQ has been reported previously (Fraga et al. 2012). However, the group receiving the conventional PZQ treatment presented just a 1.46-fold increase in glucose concentration, while the group treated with PZQ:P-407 nanosuspension (NS-2) exhibited a 7.25 fold increase. In addition, also the pyruvate concentration was elevated 110-fold in the NS-2 group. These findings could be related with the increased solubility of PZQ by means of P-407 and, therefore, an improved absorption of the drug by the target cells (Yan et al. 2010). The increased glucose, pyruvate and lactate concentrations indicate that incorporation of PZQ into nanosuspensions lead to elevated anaerobic glycolytic activity in *T. crassiceps* cysticerci compared to the non-treated

control and the groups exposed to conventional PZQ treatment. The influence of nanosuspensions in the glycolytic pathway leading to intracellular acidosis has been described in the study of multifunctional albumin-MnO_2 nanoparticles on tumor microenvironment (Prasad et al. 2014). Also the selective inhibition of a glycolytic enzyme in *Plasmodium falciparum* by polyvinylpyrrolidone-stabilized silver nanoparticles has been described by De Moor et al. (2015). Furthermore the metabolic effects of nanoparticles have been reported in protozoan parasites such as *Leishmania tropica* and *L. infantum* (Allahverdiyev et al. 2013), *P. falciparum* (Attasart et al. 2016), and in helminthes such as *Brugia malayi* causing lymphatic filariasis (Singh et al. 2016) and *Echinococcus granulosus* causing hydatid disease (Ahmadnia et al. 2013).

A partial reversion of the TCA cycle could be detected due to the absence or decrease in citrate and alfa-ketoglutarate levels, as observed in the NS-1 (PZQ:P-188) and NS-2 (PZQ:P-470) treated groups. This fumarate reductase pathway leads to the mitochondrial production of succinate from the conversion of pyruvate into malate, malate into fumarate and fumarate into succinate. This sequence of reactions may be used in the electron transport chain, or may be excreted as reported previously in *T. crassiceps* cysticerci (Leandro et al. 2014). As already observed in the glycolysis evaluation, the NS-1 and NS-2 treated groups presented significant differences in the production of organic acids of this pathway when compared to the PZQ treated group and the control group reinforcing that these formulations present a greater impact in the cysticerci metabolism than the conventional PZQ formulation. The non-detection of acetate in the PZQ, P407 and NS-1, NS-2, and NS-3 treated groups may have occurred due to the use of acetyl-CoA in the acetoacetate and beta-hydroxybutirate production, which were detected in those groups. This pathway represents an alternative for energy production, which is activated especially upon impairment of glucose uptake due to

blockage of glucose transporters or due to tegument damage, both of which may be observed during exposure to PZQ. The influence of PZQ on the urea cycle has been previously described in *T. crassiceps in vitro* and *in vivo* (Vinaud et al 2007; Fraga et al 2012). The increase in urea levels by the parasite also reflects activation of an alternative pathway for energy production due to the use of amino acids as energy source. Urea production was increased ($p<0.05$) in some of the treated groups (P188, P407 and NS-2) when compared to the control and PZQ treated groups. Interestingly in the PZQ treated group the urea production was not increased when compared to the control group. In agreement with the obtained results it should be mentioned that the stabilizers affect the parasite metabolism but are unable to eliminate the infection, while the combination of both effects are able to impair the parasite metabolism and eliminate the infection, as reported by other authors in the evaluation of Amphotericin B-P188 formulation against an AmB *Leishmania donovani* resistant line (Espuelas et al., 2000). In particular, the influence of the stabilizers might be attributed to their physical characteristics and the resulting interactions with the parasite. The choice of surfactants used in the stabilization of nanosuspensions may raise some safety concerns due to the adsorption onto cell membranes. In this context, a recent work (Ezz Eldin et al., 2014) showed some lethal effect of other non-ionic surfactants (Tween 20, 80 and Triton X-100) on *Acanthamoeba* cysts, a protozoan parasite that may cause sight-threatening keratitis. Also, Duarte et al (2009) studied the cytotoxicity of organic solvents and surfactant agents on the flagellate protozoan *Trichomonas vaginalis*, the etiologic agent of trichomoniasis, a sexually transmitted disease (STD). It was found that Tween 20, 80 and Triton X-100 presented cytotoxic effect against *T. vaginalis.*

CONCLUSIONS

In conclusion, the influence of three PZQ nanosuspensions and their respective stabilizers on the energetic metabolism of cysticerci inoculated intraperitoneally into BALB/c mice was established. The PZQ nanosuspensions increased glycolytic activity in *T. crassiceps* cysticerci compared to the groups treated with conventional PZQ and the untreated control group. Treatments with NS-1 (PZQ:P-188) and NS-2 (PZQ:P470) lead to partial reversion of the TCA cycle and activation of alternative energy production pathways, which confirms the hypothesis that these novel PZQ-nanoformulations present a greater impact on *T. crassiceps* cysticerci metabolism than the conventional PZQ formulation, probably due to a synergistic effect of the nanoformulation components. These findings also suggest that novel PZQ-nanoformulations could be highly relevant for the treatment of infections caused by other *Taenia* species and/or *Echinococcus* sp., and further studies should investigate to what extent the metabolic impact finally affects the morphology, ultrastructure, viability and infectivity of the parasites.

FINANCIAL SUPPORT: This study was financed by CNPq (Conselho Nacional de Desenvolvimento Cientifico e Tecnologico, process number 471009/2013-0), by UNR (National University of Rosario, Argentina), by CONICET (National Council Research, Argentina), and by ANPCYT (National Agency of Science and Technology, Argentina, PICT 1078). E. C. Arrúa acknowledges ANPCYT for fellowship grant.

REFERENCES

Ahmadnia, S., Moazeni, M., Mohammadi-Samani, S. and Oryan, A. (2013). In vivo evaluation of the efficacy of albendazole sulfoxide and albendazole sulfoxide loaded

solid lipid nanoparticles against hydatid cyst. *Experimental Parasitology* **135,** 314-319. doi:10.1016/j.exppara.2013.07.017

Allahverdiyev, A.M, Abamor, E.S., Bagirova, M., Baydar, S.Y., Ates, S.C., Kaya, F., Kaya, C. and Rafailovich, M. (2013). Investigation of antileishmanial activities of Tio2@Ag nanoparticles on biological properties of L. tropica and L. infantum parasites, in vitro. *Experimental Parasitology* **135,** 55-63. doi:10.1016/j.exppara.2013.06.001

Attasart, P., Boonma, S., Sunintaboon, P., Tanwilai, D., Pothikasikorn, J. and Noonpakdee, W.T. (2016). Inhibition of *Plasmodium falciparum* proliferation in vitro by double-stranded RNA nanoparticle against malaria topoisomerase II. *Experimental Parasitology* **164,** 84-90. doi:10.1016/j.exppara.2016.03.008

Budijono, S.J., Russ, B., Saad, W., Adamson, D.H. and Prud'homme, R.K. (2010). Block copolymer surface coverage on nanoparticles. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **360**, 105-110. doi:10.1016/j.colsurfa.2010.02.016.

Cioli, D., Pica-Mattoccia, L., Basso, A. and Guidi, A. (2014). Schistosomiasis control: praziquantel forever? *Molecular and Biochemical Parasitology* **195**, 23-29. doi: 10.1016/j.molbiopara.2014.06.002.

Chai, J. Y. (2013). Praziquantel treatment in trematode and cestode infections: an update. *Infection and Chemotherapy* **45**, 32-43. doi: 10.3947/ic.2013.45.1.32.

De Moor, W., van Marwijk, J., Wilhelmi, B.S. and Whiteley, C.G. (2015). Interaction of Silver Nanoparticles with Triosephosphate Isomerase from Human and Malarial Parasite (Plasmodium falciparum): A Comparative Study. *Journal of Biomedical Nanotechnology* **11,** 1071-1079. doi: http://dx.doi.org/10.1166/jbn.2015.2003

Del Brutto, O. H. (2014). Clinical management of neurocysticercosis. *Expert review of neurotherapeutics* **14** 389-396. doi: 10.1586/14737175.2014.890892.

Dong, D., Wang, X., Wang, H., Zhang, X., Wang, Y. and Wu, B. (2015). Elucidating the in vivo fate of nanocrystals using a physiologically based pharmacokinetic model: a case study with the anticancer agent SNX-2112. *International Journal of Nanomedicine* **10**, 2521-2535. doi: 10.2147/IJN.S79734

Duarte, M., Giordani, R. B., De Carli, G. A., Zuanazzi, J. A., Macedo, A. J., Tasca, T. (2009). A quantitative resazurin assay to determinate the viability of Trichomonas vaginalis and the cytotoxicity of organic solvents and surfactant agents. *Experimental Parasitology* **123**, 195-198. doi: 10.1016/j.exppara.2009.07.002.

Espíndola, N. M., Vaz, A. J., Pardini, A. X. and Fernandes, I. (2002). Excretory/secretory antigens (ES) from in-vitro cultures of *Taenia crassiceps* cysticerci, and use of an anti-ES monoclonal antibody for antigen detection in samples of cerebrospinal fluid from patients with neurocysticercosis. *Annals of Tropical Medicine & Parasitology* **96**, 361-368. doi http://dx.doi.org/10.1179/000349802125001140.

Espuelas, S,, Legrand, P., Loiseau, P. M., Bories, C., Barratt, G., Irache, J. M. (2000). In vitro reversion of amphotericin B resistance in Leishmania donovani by poloxamer 188. *Antimicrobial Agents Chemotheraphy* **44**, 2190-2192. doi:10.1128/AAC.44.8.2190-2192.2000.

Ezz Eldin, H. M., Sarhan, R. M. (2014). Cytotoxic effect of organic solvents and surfactant agents on Acanthamoeba castellanii cysts. *Parasitology Research* **113**:1949-1953. doi: 10.1007/s00436-014-3845-5.

Fraga, C. M., Costa, T. L., Bezerra, J. C. B., Lino Junior, R. S., Vinaud, M. C. (2012). *Taenia crassiceps*: Host treatment alters glycolisis and tricarboxilic acid cycle

in cysticerci. *Experimental Parasitology* **130**, 146-151. doi: 10.1016/j.exppara.2011.11.001.

Grimes, J. E. T., Croll, D., Harrison, W. E., Utzinger, J., Freeman, M. C. and Templeton, M. R. (2015). The roles of water, sanitation and hygiene in reducing schistosomiasis: a review. *Parasites & Vectors* **8**, 156-172. doi:10.1186/s13071-015-0766-9

Junghanns J. U. A. H., Müller R. H. (2008). Nanocrystal technology, drug delivery and clinical appications. *International Journal of Nanomedicine* **3**, 295-310.

Leandro, L. A., Fraga, C. M., Lino Junior, R. S. and Vinaud, M. C. (2014). Partial reverse of the TCA cycle is enhanced in *Taenia crassiceps* experimental neurocysticercosis after in vivo treatment with anthelminthic drugs. *Parasitology Research* **113**, 1313-1317. doi: 10.1007/s00436-014-3770-7.

Lee, J., Choi, J.-Y, Park, C. H. (2008). Characteristics of polymers enabling nano-comminution of water insoluble drugs. *International Journal of Pharmaceutics* **355**, 328-336. Doi: 10.1016/j.ijpharm.2007.12.032.

Mainardes, R. M., Chaud, M. V., Gremião, M. P. D. and Evangelista, R. C. (2006). Development of praziquantel-loaded PLGA nanoparticles and evaluation of intestinalpermeation by the everted gut sac model. *Journal of Nanoscience and Nanotechnology* **6**, 3057-3061. doi: http://dx.doi.org/10.1166/jnn.2006.487

Merisko-Liversidge, E. and Liversidge, G. G. (2011). Nanosizing for oral and parenteral drug delivery: A perspective on formulating poorly-water soluble compounds using wet media milling technology. *Advanced Drug Delivery Reviews* **63**, 427-440. doi:10.1016/j.addr.2010.12.007

Müller, R. H., Jabos, C. and Kayser, O. (2001). Nanosuspensions as particulate drug formulations in therapy. Rationale for development and what we can expect for the

future. *Advanced Drug Delivery Reviews* **47**, 3-19. doi:10.1016/S0169-409X(00)00118-6

Ntoukas, V., Tappe, D., Pfütze, D., Simon, M. and Holzmann, T. (2013) Cerebellar cysticercosis caused by larval *Taenia crassiceps* tapeworm in immunocompetent woman, Germany**.** *Emergent Infection Diseases* **12**, 2008-2011. doi: 10.3201/eid1912.130284

Palomares, F., Palencia, G., Pérez, R., Gonzáles-Esquivel, D., Castro, N. and Cook, H. J. (2004). In vitro effects of albendazole sulphoxide and praziquantel against *Taenia solium* and *Taenia crassiceps* cysts. *Journal of Antimicrobial Chemotherapy* **48**, 2302-2304. doi: 10.1128/AAC.48.6.2302-2304.2004.

Prasad, P., Gordijo, C.R., Abbasi, A.Z., Maeda, A., Ip, A., Rauth, A.M., daCota, R.S. and Wu, X.Y. (2014). Multifunctional Albumin–MnO2 Nanoparticles Modulate Solid Tumor Microenvironment by Attenuating Hypoxia, Acidosis, Vascular Endothelial Growth Factor and Enhance Radiation Response. *ACS Nano* **8**, 3202–3212. doi: 10.1021/nn405773r

Reich, G. (1997). In vitro stability of poly(D,L-lactide) and poly(D,L-lactide)/poloxamer nanoparticles in gastrointestinal fluids. *Drug Development and Industrial Pharmacy*, **23**, 1191-1198. doi: 10.3109/03639049709146156.

Rowe, R. C., Sheskey, P.J., Cook, W.G. and Fenton, M.E. (2009). *Handbook of Pharmaceutical Excipients*. Pharmaceutical Press; 6 edition.

Secor, W. E. (2015). Early lessons from schistosomiasis mass drug administration programs. *F1000 Research* **4**. pii: F1000 Faculty Rev-1157. doi: 10.12688/f1000research.6826.1

Singh, S. (2010). Nanomedicine-nanoscale drugs and delivery systems. *Journal of Nanoscience and Nanotechnology* **10**, 7906-7918. doi: http://dx.doi.org/10.1166/jnn.2010.3617

Singh, Y., Srinivas, A., Gangwar, M., Meher, J.G., Misra-Bhattacharya, S. and Chourasia, M.K. (2016). Subcutaneously Administered Ultrafine PLGA Nanoparticles Containing Doxycycline Hydrochloride Target Lymphatic Filarial Parasites. *Molecular Pharmaceutics* **[epub ahead of print].** doi: 10.1021/acs.molpharmaceut.6b00206

Tielens, A. G. M. and Van Hellemond, J. J. (2005). Unusual aspects of metabolism in flatworm parasites. In: Maule AG, Marks NJ. Parasitic Flatworms: Molecular Biology, Biochemistry, Immunology and Physiology. CABI editor. London, UK. p. 387-407.

Utzinger, J. and Keiser, J. (2004). Schistosomiasis and soil-transmitted helminthiasis: common drugs for treatment and control. *Expert Opinion on Pharmacotherapy* **2**, 263-285. doi:10.1517/14656566.5.2.263

Vinaud, M. C., Lino Junior, R. S. and Bezerra, J. C. B. (2007). *Taenia crassiceps* organic acids detect in cysticerci. *Experimental Parasitology* **116**, 335-339. doi:10.1016/j.exppara.2007.01.013

Vinaud, M. C., Ferreira, C. S., Lino Junior, R. S. and Bezerra, J. C. B. (2008). *Taenia crassiceps*: Energetic and respiratory metabolism from cysticerci exposed to praziquantel and albendazole in vitro. *Experimental Parasitology* **120**, 221-226. doi:10.1016/j.exppara.2008.07.008

Vinaud, M. C., Ferreira, C. S., Lino Junior, R. S. and Bezerra, J. C. B. (2009). *Taenia crassiceps*: fatty acids ocidation and alterantive energy source in *in vitro* cysticerci exposed to antihelminthic drugs. *Experimental Parasitology* **122**, 208-211. doi:10.1016/j.exppara.2009.03.015

Xie, S., Pan, B., Wang, M., Zhu, L., Wang, F., Dong, Z., Wang, X. and Zhou, W. (2010). Formulation, characterization and pharmacokinetics of praziquantel-loaded hydrogenated castor oil solid lipid nanoparticles. *Nanomedicine* **5**, 693-701. doi:10.2217/nnm.10.42.

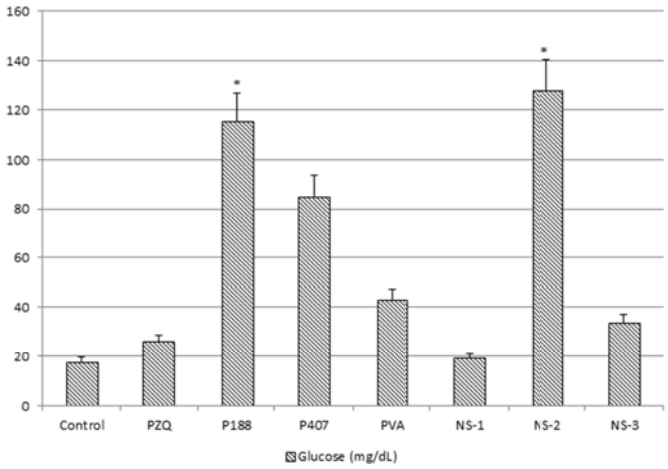


Figure 1. Modification of glucose concentrations in *T. crassiceps* cysticerci treated with control, raw PZQ, Poloxamer 188 (P188), Poloxamer 407 (P407), Poly(vinyl) alcohol (PVA), and PZQ nanosuspension NS-1, NS-2, and NS-3.

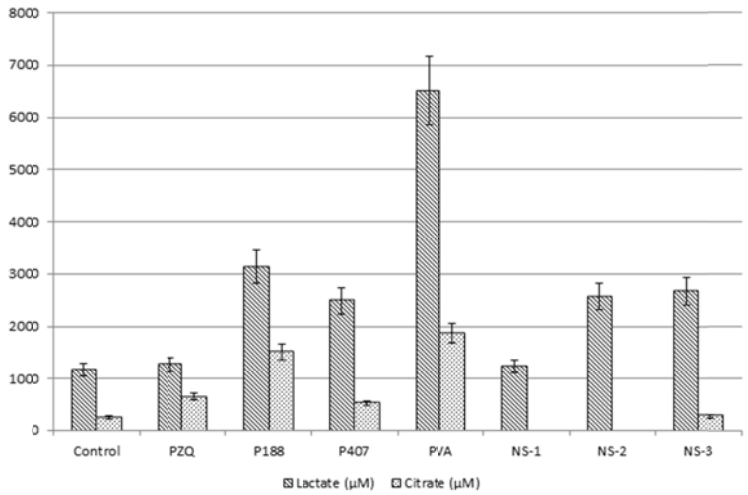


Figure 2. Modification of Lactate and Citrate concentrations in *T. crassiceps* cysticerci treated with control, raw PZQ, Poloxamer 188 (P188), Poloxamer 407 (P407), Poly(vinyl) alcohol (PVA), and PZQ nanosuspension NS-1, NS-2, and NS-3.

Table 1. Glycolisis organic acids, glucose, pyruvate and lactate production in *Taenia crassiceps* cysticerci *in vivo* exposed to different treatments.

	Control	PZQ	P188	P407	PVA	NS-1	NS-2	NS-3
Glucose (mg/dL)	17.6 ±4.72	25.75 ± 15.45	**115 ± 42.57***	85 ± 7.25	43 ± 9.89	19 ± 4	**127.66 ± 22.5***	33.4 ± 14.77
Pyruvate (µM)	1.20 ±0.77	61.29 ± 23.52	29.847 ± 14.446	15.84 ± 9.75	31.72 ± 29.88	**101.82 ± 24.42***	**132.01 ± 24.48***	ND
Lactate (µM)	1164.21 ± 262.89	1265.6 ± 607.07	3140.44 ± 1963.36	2488.91 ± 919.85	**6525.72 ± 1635.39***	1234.06 ± 397.52	2571.40 ± 429.47	2664.92 ± 1820.66

ND - non detected; Bold and * - $p<0.05$ (ANOVA); PZQ (*praziquantel treated group*); P188 (*group treated with P188*); P407 (*group treated with poloxamer 407*); PVA (*group treated with polyvinyl alcohol*); NS-1 (group treated with PZQ-P188 nanosuspension); NS-2 (group treated with PZQ-P407 nanosuspension); NS-3 (group treated with PZQ-PVA nanosuspension).

Table 2.Tricarboxilic acid cycle organic acids (μM) production in *Taenia crassiceps* cysticerci *in vivo* exposed to different treatments.

	Control	PZQ	P188	P407	PVA	NS-1	NS-2	NS-3
Citrate	251.38 ± 45.1	**646.87 ± 87.53***	**1514.49 ± 388.31***	**527.38 ± 367.21***	**1867.25 ± 684.0***	ND	ND	**278.76 ± 125.79***
α-kg	24.42 ± 16.67	ND	ND	ND	ND	ND	ND	ND
OAA	7.09 ± 2.15	65.92 ± 33.32	**149.25 ± 75.52***	49.69 ± 71.79	**243.61 ± 53.51***	74.66 ± 24.53	**228.95 ± 55.05***	**8.18 ± 4.56***
Malate	698.67 ± 161.51	ND	1984.55 ± 368.94	1153.17 ± 826.48	**1643.5 ± 430.88***	ND	ND	**601.79 ± 242.96***
Fumarate	136.48 ± 11.65	155.67 ± 47.09	**394.03 ± 120.87***	266.25 ± 164.18	**293.54 ± 37.08***	186.99 ± 42.67	233.90 ± 13.93	**101.41 ± 83.76***
Succinate	453.91 ± 148.10	1109.76 ± 644.63	**5135.55 ± 1960.40***	2455.47 ± 1094.24	ND	**1211.15 ± 284.83***	**3938.02 ± 815.99***	ND

α-kg- Alfa-ketoglutarate; OAA – Oxaloacetate; ND - non detected; Bold and * - $p<0.05$ (ANOVA). PZQ (*praziquantel treated group*); P188 (*group treated with P188*); P407 (*group treated with poloxamer 407*); PVA (*group treated with polyvinyl alcohol*); NS-1 (group treated with PZQ-P188 nanosuspension); NS-2 (group treated with PZQ-P407 nanosuspension); NS-3 (group treated with PZQ-PVA nanosuspension).

Table 3. Ketonic bodies (μM), urea (mg/dL) and creatinine (mg/dL) production in *Taenia crassiceps* cysticerci *in vivo* exposed to different treatments.

	Control	PZQ	P188	P407	PVA	NS-1	NS-2	NS-3
Acetate	333.810 ± 147.062	ND	**1547.978 ± 387.405***	ND	**1855.02 ± 574.11***	ND	ND	ND
Acetoacetate	108.944 ± 23.723	330.570 ± 185.836	ND	360.945 ± 169.573	ND	336.182 ± 159.046	**628.031 ± 51.995***	ND
BHB	2707.371 ± 1256.930	1537.078 ± 594.231	10521.196 ± 2623.888	6400.530 ± 1610.638	4164.46 ± 2887.11	1160.358 ± 1417.105	5702.844 ± 1308.746	1893.551 ± 240.615
Fumarate	136.48 ± 11.65	155.67 ± 47.09	**394.03 ± 120.87***	266.25 ± 164.18	**293.54 ± 37.08***	186.99 ± 42.67	233.90 ± 13.93	**101.41 ± 83.76***
Urea	11 ± 1	14.5 ± 7.9	**28.3 ± 6.6***	**30.5 ± 0.6***	20.5 ± 3.10	10.6 ± 2.5	**34.6 ± 1.5***	19 ± 8.8
Creatinine	0.2 ± 0.001	0.22 ± 0.09	0.16 ± 0.05	0.2 ± 0.001	0.17 ± 0.05	0.13 ± 0.05	0.13 ± 0.05	0.14± 0.05

BHB - Beta-hydroxybutyrate; ND - non detected; Bold and * - $p<0.05$ (ANOVA). PZQ (*praziquantel treated group*); P188 (*group treated with P188*); P407 (*group treated with poloxamer 407*); PVA (*group treated with polyvinyl alcohol*); NS-1 (group treated with PZQ-P188 nanosuspension); NS-2 (group treated with PZQ-P407 nanosuspension); NS-3 (group treated with PZQ-PVA nanosuspension).