Drug Delivery and Translational Research
https://doi.org/10.1007/s13346-018-0576-7

ORIGINAL ARTICLE

In vivo treatment of experimental neurocysticercosis with praziquantel nanosuspensions—a metabolic approach

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Abstract

Neurocysticercosis is the most common parasitic infection of the nervous system and currently represents a serious public health issue in many regions of Latin America, Asia, and Africa. To date, praziquantel is one of the chosen drugs for the treatment of neurocysticercosis. Its mechanism of action is based on the inhibition of different biochemical pathways within the parasite which contribute to its death. Thus, the aim of this work was to analyze, for the first time, whether the nanoformulations of praziquantel would modify the energetic pathway of *Taenia crassiceps* cysticerci, after an intracranial inoculation in BALB/c mice. Praziquantel nanosuspensions were formulated with polyvinyl alcohol, poloxamer 188, and poloxamer 407, as stabilizers. These formulations exhibited particle size in a range of 74–285 nm and zeta potential values in a range of − 8.1/− 13.2 depending on the type of stabilizer. Physical stability study at both 4 ± 2 and 25 ± 2 °C indicated that praziquantel (PZQ) nanoparticles were stable in terms of solubility and particle size after 120-day storage. In vivo studies demonstrated that those nanosystems were able to produce significant modifications on the concentrations of oxaloacetate, citrate, pyruvate, alpha-ketoglutarate, malate, succinate, lactate, beta-hydroxybutyrate, fumarate, and propionate involved in the metabolism of *Taenia crassiceps* cysticerci. Therefore, these nanoformulations may be considered as a promising tool to deliver praziquantel to the brain for the effective management of neurocysticercosis.

Keywords *Taenia crassiceps* · Neurocysticercosis · In vivo · Praziquantel · Nanotechnology

Introduction

To date, parasitic infections are mainly affecting vulnerable communities in developing countries by lack of clean water and sanitation. In this context, malaria, tuberculosis, Chagas disease, leishmaniasis, toxocariasis, toxoplasmosis, and trichomoniasis are considered neglected diseases based on the amount of people infected, the severity of the illnesses, and the existing tools to prevent and treat them. In particular, cysticercosis, an infection caused by the larval form of the pork tapeworm, *Taenia solium* (*T. solium*), has been recently classified by the World Health Organization (WHO) as a "major neglected disease" [1]. Even though it is affecting poor countries and regions, lately this infection has been detected in developed regions including North America, Europe, and Japan [2]. The adult tapeworm develops only in the intestine of a human host, after ingestion of raw or poorly cooked infected pork. Neurocysticercosis (NCC) is also an endemic infection in many regions caused by the migration of the cysticerci (encysted tapeworm larvae) from the intestine to the central nervous system. NCC is the leading cause of acquired epilepsy in low- and median-income countries. It is also the most frequent preventable helminth infection of the central nervous system [3]. In addition, it is responsible for one-third of seizure disorders and an estimation of 2 million affected people in the developing world. Therefore, it places a significant economic burden on communities and society [4].

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Published online: 16 August 2018

One of the available solutions to treat NCC is the surgical removal of the cysticercus. However, in many cases, such alternative may lead to death due to later complications [5].

Praziquantel (PZQ) is one of the widely used chemotherapeutic agents for the treatment of NCC [6]. It is supposed to be active in controlling seizures in affected patients, acting against active forms of the infection and in patients with low risk of increased intracranial pressure [7]. However, it is poorly soluble in water and, as a consequence, exhibits a low absorption process and an erratic oral bioavailability after being orally administered [8]. In addition, PZQ presents a high hepatic first-pass metabolism and, therefore, high doses are needed to produce the desired antiparasitic effects [9]. In this respect, novel chemotherapeutic alternatives are urgently needed to avoid such drawbacks.

The ability of reducing the drug particle size to a nanometer scale is an attractive approach extensively applied to improve aqueous solubility, dissolution rate, and bioavailability of hydrophobic molecules [10, 11]. In particular, nanonization of PZQ has become one of the most attractive approaches to improve drug delivery and release at the target sites in a proper dose reducing, as a consequence, the toxicity and side effects. Different nanoformulations including liposomes [12], solid lipid nanoparticles [13], and nanoemulsions [14] were successfully developed to improve both the physicochemical and biopharmaceutical properties of PZQ. However, nothing is found in the literature about the effects of nanoformulated PZQ on the metabolism of the parasite after infecting the central nervous system of the host. As previously stated, the analysis of the modification of the metabolic pathway on parasites is possible to determine both novel therapeutical targets and mode of actions [15]. Thus, the impact of PZQ nanosuspensions on metabolism of cysticerci inoculated intraperitoneally into BALB/c mice was well established [16]. It was demonstrated that nanoformulated PZQ significantly modified the *T. crassiceps* cysticerci metabolism compared to untreated drug. Due to this, larval cysts may migrate to the brain and produce several neurological disorders including epilepsy, and the effects of PZQ nanosuspensions over the metabolism of *T. crassiceps* cysticerci after an intracranial inoculation in BALB/c mice, used as a model host, were herein investigated. The preparation of the PZQ nanocrystals was performed through precipitation approach (bottom-up technique) using three different excipients as stabilizers [17]. Solubility and dissolution studies of the nanosystems were evaluated. Then, a long-term stability study (120 days) at 4 ± 2, 25 ± 2, and 50 ± 2 °C was also performed [18]. The concentrations of organic acids (oxaloacetate; citrate; pyruvate; alpha-ketoglutarate; malate; succinate; lactate; beta-hydroxybutyrate; fumarate, and propionate) involved in the metabolism of *T. crassiceps* cysticerci after treatment with those PZQ nanosuspensions using spectrophotometric and high-performance liquid chromatography (HPLC) analysis were investigated. To our knowledge, this is the first report regarding the impact of nanoformulated PZQ on the metabolism of *T. crassiceps* cysticerci in neurocysticercosis.

Materials

PZQ 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 purposes were of chromatography grade.

Methods

Formulation of PZQ nanosuspensions

PZQ nanosuspensions were prepared by the solvent diffusion method following the procedure previously described [16]. Briefly, 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 each stabilizer (P188, P407, or PVA) under magnetic stirring (500 rpm). The resulting emulsion was then stirred (500 rpm) for 18 h at room temperature to allow solvent evaporation. PZQ nanocrystals stabilized with P188 (NS-1), P407 (NS-2), and PVA (NS-3) were recovered by centrifugation using a MIKRO 220 Hettich centrifuge (Andreas Hettich GmbH & Co.KG, Tuttlingen, Germany) for 20 min (15,000 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 °C and freeze-dried (48 h).

Lyophilization of PZQ nanosuspensions

PZQ nanosuspensions were freeze-dried by means of a Labconco FreeZone 4.5 l (Labconco, Kansas City, MO, USA) for 48 h at − 40 °C. The obtained samples were diluted to original volume with distilled water [19].

Particle size determination

BNZ-nps particle size was determined by experiments using dynamic light scattering at a scattering angle of 90 to 25 °C using a nanoparticle analyzer SZ-100. The parameters measured were the polydispersity index (PDI), z-average

diameter, and zeta potential (ζ). In evaluating the particle size, the intensity distribution was used and, therefore, the z-average diameter is an intensity mean diameter, and the PDI describes the width of the particle size distribution. The median-volume particle size, *d* (*v*, 0.5) (size of the particles for which 50% of the sample volume contains particles smaller than '*d* 0.50, the other particles being larger than '*d* 0.50), *d* (*v*, 0.1), *d* (*v*, 0.9), and the volume mean diameter *D* [3, 4] were used as characterization parameters. Nanocrystal solutions were prepared in a solution of Tween 20 (0.1% *w*/*v*), previously filtered. Two transparent face cuvettes were used. The sample contained no more than 0.01% by volume of particles. The measurements were performed in triplicate and the data obtained was expressed as mean ± SD.

Saturation solubility studies

The saturated solubility of PZQ and PZQ nanocrystals was calculated by adding an excess amount (100 mg) of each sample in a vial with 5 ml of solution of medium (distilled water, pH 6.3). The samples were shaken in a Boeco orbital shaker (Boeckel + Co, Hamburg, Germany) at 25 °C and 150 rpm until equilibrium was reached (72 h). Upon equilibrium, the samples were filtered through a 0.45-μm filter and measured by UV at 260 nm using a Boeco UV-Vis spectrophotometer (Boeckel + Co, Hamburg, Germany) [20]. All experiments were carried out in triplicate.

Dissolution studies

In vitro dissolution studies were carried out according to the USP-30 by means of paddle method at 50 rpm (SR8 8-Flask Bath, Hanson Research, Chatsworth, CA) using, as dissolution media (37 ± 0.5 °C), a mixture of 0.1 N HCl/2.0 mg/ml of sodium dodecyl sulfate (SDS). Samples of raw PZQ, NS-1, NS-2, and NS-3 equivalent to 50 mg of the drug were added to the dissolution medium. At different time intervals, 5 ml of samples was withdrawn and filtered (pore size 0.45 mm). PZQ concentration was determined by UV as described in the "Saturation solubility studies" section. It was found that the stabilizers did not interfere with the analysis of the samples at 260 nm. The dissolution efficiency (DE), a parameter which characterizes drug release [21, 22] and is defined as the area under a dissolution curve between specified time points, was calculated using the following equation:

$$\text{DissolutionEfficiency}\%(\text{DE}) = \frac{\int_o^t y \times dt}{y_0 \times 100} \times 100$$

where *y* is the percentage of dissolved product at time *t*. The results presented are mean values of three determinations.

Physical stability studies

The stability study was performed to evaluate the stability of PZQ nanosuspensions, under controlled conditions of temperature (4 ± 2, 25 ± 2, and 50 ± 2 °C). The samples were stored in closed vials, protected from light, in a refrigerator, in a close cupboard, and in a pharmaceutical stability chamber (PH09 chamber, Darwin Chambers Company, St. Louis, MO, USA). NS-1, NS-2, and NS-3 samples were characterized for solubility and particle size at 0, 7, 14, 30, 61, and 120 days, respectively. All determinations were carried out in triplicate and the mean values and standard deviations are reported.

Mice infection and treatment

T. crassiceps (ORF strain) cysticerci are maintained in the animal facility of the Tropical Pathology and Public Health Institute from the Federal University of Goiás (IPTSP/UFG) as described previously [23, 24]. For this study, we use animals from 8 to 12 weeks old and with 20 to 30 g of weight. These animals were intracranially inoculated with three to five initial stages of cysticerci as described by Matos-Silva et al. [24]. The animals were divided into five groups containing five animals each, as follows: group 1, BALB/c mice inoculated with cysticerci treated with physiological solution (NaCl 0.9%); group 2, BALB/c mice inoculated with cysticerci and treated with 50 mg/kg of PZQ; group 3, BALB/c mice inoculated with cysticerci and treated with 50 mg/kg of NS-4; group 4, BALB/c mice inoculated with cysticerci and treated with 50 mg/kg of NS-5; and group 5, BALB/c mice inoculated with cysticerci and treated with 50 mg/kg of NS-6. All animals were treated after 30 days post-inoculation as to insure the infection and were euthanized 24 h after the treatment. All mice were weighed before and 24 h after the treatment. All the experiments were performed in five independent repetitions. The ethical principles for animal experimentation professed by the Brazilian Society of Laboratory Animal Sciences (Sociedade Brasileira de Ciência em Animais de Laboratório (SBCAL)) were followed, and this study was authorized by the Ethics Committee for Animal Use (CEUA/UFG) (registration number 050/13). This study was performed following the recommendations for the use of laboratory animals (World Medical Association in the Declaration of Helsinki), also. The mice received daily care, acidified water, and standard rations.

Cysticerci biochemical analysis

The cysticerci removed from the mice were washed with saline solution, fixed in liquid nitrogen, weighted, and homogenized with 500 μl of tris-HCl 0.1 M buffer supplemented with a protease inhibitor (SigmaFast protease inhibitor cocktail tablets, EDTA-free, Sigma®), pH 7.6 [25]. The extract

obtained was centrifuged at 10,000 rpm per 10 min at 4 °C and the organic acids were extracted for chromatographic analysis and identified according to the previously determined retention time and calibration, as already described [15, 26]. The organic acids analyzed were the ones which indicate the energetic metabolism such as pyruvate, citrate, oxaloacetate, malate, fumarate, succinate, α-ketoglutarate, acetate, acetoacetate, β-hydroxybutyrate, and propionate. Their concentrations were calculated in nanomolars of organic acid per gram of cysticerci, as the number of cysticerci removed from each mouse was not uniform.

Statistical analysis

The statistical analysis was performed using the SigmaStat 3.5 program. 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. As they presented normal distribution, variance analysis was used. The differences noted were considered significant when $p < 0.05$.

Results and discussion

To date, PZQ is the drug of choice for the treatment of several neglected parasitic diseases [6]. It is poorly water-soluble (class II of the Biopharmaceutical Classification System) and, therefore, high doses are commonly required to achieve tissue larval parasite. Moreover, due to its lipophilic properties, the development of proper formulation of PZQ for oral administration to overcome the challenges of low absorption and poor bioavailability is still urgently needed [27]. Thus, in this work, PZQ was formulated as a nanosuspension, a submicron colloidal dispersion, with the addition of P188 (NS-1), P407 (NS-2), or PVA (NS-3) as stabilizers. PZQ nanocrystals were obtained through the nanoprecipitation single-step process at room temperature [16]. PZQ was dissolved in ethanol (solvent phase) and then added to an aqueous solution (antisolvent phase) containing the corresponding stabilizer. The ratio of ethanol:water and the magnetic stirring were kept constant during the preparation of all the samples. After 5 min, the appearance of turbidity was seen, probably due to a supersaturation phenomenon, leading to a fast nucleation and further precipitation of PZQ nanocrystals [28]. As reported, the type and concentration of stabilizer play a crucial role during particle size reduction process leading to the production of nanoparticles with acceptable physicochemical properties. Particularly, Pluronics® is able to induce crystallization of several hydrophobic molecules acting as templates or seeds to generate a faster nucleation of the drug particles [29]. In this study, P188 and P407 yielded PZQ nanocrystals of 74.6 ± 4.2 nm and 282 ± 1.9 nm, respectively, while PVA produced particles of 105.4 ± 2.4 nm. PZQ formulated with P188, P407, and PVA exhibited a zeta potential of − 8.1, − 8.6, and − 13.2, with a polydispersity index (PI) of 0.19, 0.30, and 0.24, respectively (Table 1).

The obtained values of the particle size indicated a high reproducibility and the obtained polydispersity indexes indicate the formation of a homogeneous PZQ nanosuspension. The negative values of zeta potential suggest that the new samples would be stabilized through steric effects of the corresponding stabilizers, as described by Dong et al. [30]. The values of the saturation solubility study of raw PZQ and PZQ samples are seen in Table 1. Such results showed that PZQ nanocrystals exhibit a very high solubility in water compared to the raw drug. The saturation solubility of raw PZQ in distilled water was found to be 417.40 ± 1.14 mg/ml. On the other hand, NS-1 saturation solubility increased up to maximum of 4805.61 ± 1.58 mg/ml, while NS-2 and NS-3 showed a saturation solubility of 5340.54 ± 1.76 mg/ml and 6882.35 ± 1.23 mg/ml, respectively. As seen in this assay, PZQ nanocrystal saturation solubility was more than ten folds higher than that of the raw drug. This finding could be due to the reduction of drug particle size to the nanometer range after the nanoprecipitation. It should be noted that a higher saturation solubility in the lumen of the gut enhances the concentration gradient between the lumen and blood and, as a consequence, it may improve the absorption process [31].

The dissolution profiles of raw PZQ and the corresponding samples (NS-1, NS-2, and NS-3) are shown in Fig. 1. Clearly, it can be observed that nanoformulated PZQ exhibited a significant improvement in both the rate and extent of dissolution in comparison with untreated PZQ. Particularly in the first 10 min, up to 64% of PZQ nanoformulated was released while only 13% of raw drug was dissolved. At the end of the assay (180 min), raw drug presented a moderate dissolution rate (57%). In contrast, PZQ nanosystems exhibited an almost quantitative dissolution rate (> 95%) indicating the high solubilization behavior of PZQ when formulated as nanocrystals.

These findings clearly exhibit the ability of PZQ nanoformulations to improve the dissolution performance of the drug in comparison with the untreated one. It could be due to the decreased particle size and, as a consequence, an increased specific surface area, as described by the Nernst–Brunner and Noyes–Whitney equations [32]. In addition, it should be noted that nanoparticles also show increased solubility, which is due to the vapor and dissolution pressure of solid particles that increases when particle sizes decrease below 1000 nm, and more particularly below 100 nm, as described by the Freundlich–Ostwald equation [33]. The dissolution efficiency (DE), defined as the area under the dissolution curve up to a specific time period, is a suitable parameter to compare and optimize the in vitro dissolution of different pharmaceutical formulations and also to analyze their in vitro biopharmaceutical performance [34]. In addition, DE is a

Table 1 Physicochemical characterization of PZQ nanoformulations

Samples code	Stabilizer	Mean particle size (nm)	Zeta potential (−mV)	Polydispersity index	Saturation solubility (%)
NS-1	P188	74.60 ± 0.40	− 8.1 ± 0.82	0.19 ± 0.22	4805.61 ± 1.58
NS-2	P407	282.60 ± 0.22	− 8.6 ± 1.02	0.30 ± 0.79	5340.54 ± 1.76
NS-3	PVA	105.40 ± 0.53	− 13.2 ± 0.93	0.24 ± 0.90	6882.35 ± 1.23

Values are mean ± SD ($n = 3$)

useful tool to analyze the drug release data into a single table allowing, as a consequence, fast comparison between several formulations. Thus, in this study, DE values for raw PZQ and the samples, at 10, 30, 60, 90, and 120 min, were calculated. As seen in Table 2, DE values were consistent with the dissolution studies (Fig. 1), indicating that the release of nanoformulated PZQ is faster compared to the raw drug. For instance, the DE value of raw PZQ at 10 min was 13% whereas this value increased to 58% and 63% for the NS-3 and NS-1 formulations, respectively. These findings clearly show that the nanoprecipitation is a very convenient technique to reduce the particle size and to optimize the dissolution performance of PZQ.

All the PZQ nanoformulations prepared were subjected to a stability test at 4 ± 2 °C, 25 ± 2 °C, and 50 ± 2 °C for 120 days (Table 3). For each measurement, the nanosuspensions were brought to room temperature. As already described, storage conditions of a drug product may lead to several stability issues including drug precipitation and growth of crystals. In addition, the formation of aggregates leads to the reduction of drug solubility and consequently an unsuccessfully in vivo performance [28]. After storage, there was no significant change on solubility of NS-1, NS-2, and NS-3 samples. Results showed that the solubility of NS-1 samples stored at 4 ± 2 °C, 25 ± 2 °C, and 50 ± 2 °C was 4757.2 ± 1.15 mg/ml, 4703.4 ± 0.98 mg/ml, and 4516.8 ± 1.32 mg/ml, respectively, while the solubility at $t = 0$ was 4805.6 ± 1.58 mg/ml. The analysis of NS-2 showed a solubility of 5340.5 ± 1.06 mg/ml ($t = 0$) being slightly reduced after storage: 5249.1 ± 0.88 mg/ml (at 4 ± 2 °C), 5216.7 ± 1.43 mg/ml (25 ± 2 °C), and 5017.7 ± 1.43 mg/ml (50 ± 2 °C). Similarly, NS-3 exhibited a solubility of 6882.3 ± 0.93 mg/ml ($t = 0$) and 6903.5 ± 1.12 mg/ml (at 4 ± 2 °C), 6889.7 ± 0.98 mg/ml (25 ± 2 °C), and 6554.9 ± 1.46 mg/ml (50 ± 2 °C). In addition, the stability of the nanoformulations in terms of particle size was also evaluated over the time to analyze whether this process would produce

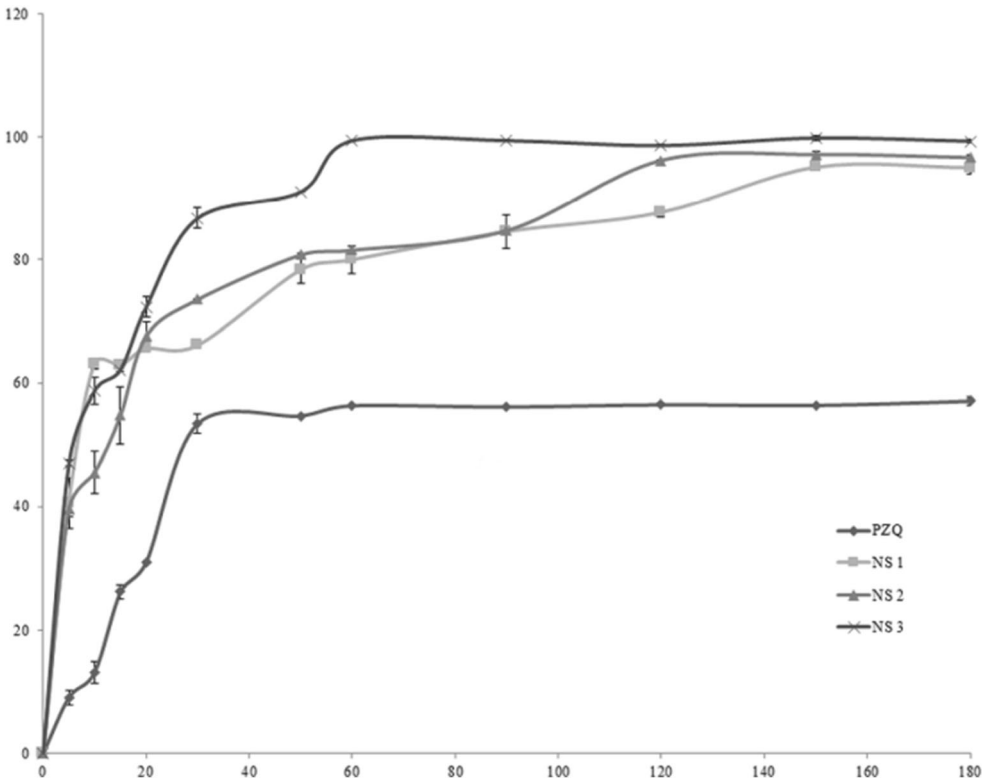

Fig. 1 Dissolution profiles of PZQ and the corresponding samples

Table 2 Dissolution efficiency values (%) of PZQ and PZQ nanoformulations

Sample	DE_{10}[a]	DE_{30}[a]	DE_{60}[a]	DE_{90}[a]	DE_{120}[a]
PZQ	13.14 ± 1.81	53.43 ± 1.55	56.35 ± 0.85	56.15 ± 0.57	56.41 ± 0.46
NS-1	63.13 ± 0.90	66.09 ± 0.28	80.12 ± 2.24	84.70 ± 2.16	87.84 ± 0.83
NS-2	45.51 ± 3.42	73.54 ± 0.90	81.55 ± 0.67	84.76 ± 0.95	96.24 ± 0.60
NS-3	58.73 ± 1.56	86.79 ± 0.98	99.45 ± 1.46	99.46 ± 0.89	98.90 ± 1.78

[a] DE_{10}, DE_{30}, DE_{60}, DE_{90}, and DE_{120} indicate dissolution efficiency at 10, 30, 60, 90, and 120 min. Each value is the average of three determinations

particles with acceptable stability. As seen in Table 3, after 120 days, it was found that the particle size of nanoformulations stored at 4 ± 2 °C and 25 ± 2 °C remained almost unchanged. A similar finding was reported by Jain et al. [18] for the formulation of optimization of olmesartan medoxomil nanocrystals using P188 as stabilizer. In another study, no significant change in terms of size was observed for trans-resveratrol nanocrystals formulated with P407, after 3 months of storage [17].

On the other hand, at 50 ± 2 °C, the particle size of the nanoformulations was increased probably due to partial agglomeration of the nanocrystals. This result is in agreement with the studies of Martena et al. [35] who demonstrated that nicergoline nanocrystals exhibited an increase of particle size after storage at 40 °C and Sahu et al. [36] who show that felodipine nanoparticles stored at 40 °C exhibited an increase in mean particle size after 6 months of storage. Taking into account these findings, it could be postulated that the stabilizers were able to avoid or reduce, at least, the crystal growth due to strong interactions with the surface of the PZQ particles [17].

As shown, PZQ is one of the two drugs of choice to treat NCC by killing around 75% of parenchymal brain cysticerci [7]. On the other hand, a significant impact of PZQ in the energy and respiratory metabolism of the parasite *T. crassiceps*, an experimental model related to both *T. solium* and *T. saginata*, has been reported [24, 37, 38]. Such model allows both pathophysiological and parasitological studies of the host–parasite relationship. Related with it, it was observed that PZQ nanosuspensions exhibited more impact on the parasite metabolism when compared to raw PZQ because they lead to the non-detection of important metabolic pathways, such as the mitochondrial ones [16]. Even though those results were very promising in terms of efficacy and reduction of infection, it should be mentioned that the most severe form of cysticercosis occurs when the central nervous system (CNS) is affected, leading to seizures, epilepsy, and death [39]. Therefore, the impact of PZQ nanoformulations over the metabolic pathway of cysticerci after intracranial infection was evaluated for the first time. Thus, all mice were intracranially inoculated with *T. crassiceps* cysticerci 30 days and treated orally with 50 mg/kg of raw PZQ and the prepared nanoformulations. Following the corresponding treatment, the oxaloacetate production was undetectable in the NS-1 and NS-2, while the treatment with NS-3 produced a similar amount of oxaloacetate than the control group treated with NaCl 0.9% solution. The non-detection of a pathway that is detected in the control group suggests that both NS-1 and NS-2 were able to modify the production of oxaloacetate in cysticerci removed from mice brain. In the case of citrate, administration of NS-1 and NS-3 did not produce it, while the treatment with NS-2 produced a similar amount of citrate than the PZQ control group. On the other hand, both NS-1 and PZQ control groups did not produce pyruvate in contrast to NS-2, NS-3, and control group. Moreover, undetectable amounts of α-ketoglutarate and succinate were observed

Table 3 Evaluation of PZQ particle size during storage (30, 60, 90, and 120 days)

Sample	Storage	0 day	30 days	60 days	90 days	120 days
NS-1	4 ± 2 °C	74.6 ± 4.2	77.1 ± 2.5	76.7 ± 4.7	75.9 ± 3.1	76.2 ± 1.8
	25 ± 2 °C	74.6 ± 4.2	75.9 ± 4.2	77.1 ± 2	76.9 ± 4.2	77.1 ± 2.7
	50 ± 2 °C	74.6 ± 4.2	77.8 ± 3	79.1 ± 1.9	78.9 ± 3.7	80.5 ± 3.6
NS-2	4 ± 2 °C	282 ± 1.9	281 ± 2.4	282.6 ± 2.1	287.2 ± 3.8	289.6 ± 1.8
	25 ± 2 °C	282 ± 1.9	286.9 ± 1.4	288.6 ± 3.1	290 ± 2	291.9 ± 2.3
	50 ± 2 °C	282 ± 1.9	292.3 ± 2.1	296.5 ± 4.8	299.4 ± 4.1	307.4 ± 3.2
NS-3	4 ± 2 °C	105.4 ± 2.4	105.9 ± 3.1	105.2 ± 1.3	104.8 ± 3.1	107.5 ± 2.8
	25 ± 2 °C	105.4 ± 2.4	105.2 ± 2.9	104.9 ± 2.9	105.1 ± 4.2	110.2 ± 4.1
	50 ± 2 °C	105.4 ± 2.4	110.8 ± 1.8	115.7 ± 2.2	135.2 ± 3	146.3 ± 3.5

Table 4 Organic acids (μM) detected per gram of cysticerci removed from the CNS of BALB/c mice after treatment with 50 mg/kg of NS-1, NS-2, NS-3, and PZQ

	Control	NS-1	NS-2	NS-3	PZQ
Oxaloacetate	2.09 ± 0.005	ND	ND	3.55 ± 0.08	ND
Citrate	56.63 ± 0.23	ND	77.21 ± 59.20	ND	59.37 ± 11.53
Pyruvate	4.94 ± 1.19	ND	8.37 ± 3.74	5.00 ± 1.48	ND
Alpha-ketoglutarate	2.36 ± 0.98	ND	ND	ND	ND
Malate	66.87 ± 1.21	ND	297.19 ± 273.12	ND	ND
Succinate	104.29 ± 31.59	ND	ND	ND	ND
Lactate	594.44 ± 23.88	4331.72 ± 3339.60	2107.88 ± 2520.38	*296.06 ± 62.92**	481.48 ± 172.55
Beta-hydroxybutyrate	447.71 ± 8.24	3619.42 ± 4325.91	ND	308.76 ± 21.97	ND
Fumarate	13.37 ± 0.13	112.28 ± 138.06	*32.44 ± 3.64**	ND	8.90 ± 8.48
Propionate	143.27 ± 106.92	5174.85 ± 3686.95	ND	ND	ND

Italics and * statistical difference ($p < 0.05$) when compared to the control group

after treatment with NS-1, NS-2, NS-3, and PZQ. Additionally, a blockage of the mitochondrial pathways after the NS-1, NS-2, and NS-3 treatments with preference for the anaerobic pathway (lactate detection) and fatty acid oxidation (beta-hydroxybutyrate and propionate detection) was observed. As seen in Table 4, NS-3 treatment induced a significant decrease in the lactate levels ($p < 0.05$). One of the alternative metabolic pathways induced by the nanosuspension treatment is the fumarate reductase pathway observed in the NS-2-treated group, which was not observed in the control group. This pathway ensures the aerobic mitochondrial metabolism and energy production by the parasite although it is not a preferential pathway used by *T. crassiceps* [40]. It should be noted that similar results were described in the case of cysticerci removed from mice brain treated with low doses of conventional PZQ and albendazole [37]. On the other hand, in the NS-4- and NS-5-treated groups, not even this pathway was activated and the parasite was forced into an anaerobic metabolism observed through the lactate detection and into the fatty acid oxidation observed through the beta-hydroxybutyrate and propionate detection. As reported, these are other alternative pathways used by cestodes as to ensure survival in hostile environments such as the ones observed when there are drugs or toxic substances [41]. In addition, the suppression of important metabolic pathways may be used as an escape mechanism as to ensure the parasite survival [42].

PZQ is known to induce tegumental damage and disruption in both *Mesocestoides corti* [43] and *Schistosoma mansoni* [44]. Such damage was described through ultrastructure analysis of the surface of metacercariae of *Brachylaima* sp. after PZQ treatment which showed alterations in the glycocalyx and in secretory glands [45]. Also, it induced destruction and vacuolization of the tegument of *Opisthorchis felineus* in in vitro and in vivo experiments [46]. Another described mode of action of PZQ is the rapid influx of Ca^{2+} [47, 48], which may be one of the causes of the metabolic alterations observed in this study. The increased intracellular concentration of Ca^{+2} induces muscle contraction in the parasite which elicits a greater energy production. All the combined effects of tegumental damage and muscle contraction lead to the biochemical impairment observed in our results. Attempts to improve PZQ efficacy have been reported through liposomal formulations [49]. Unfortunately, liposomized PZQ decreased its efficacy when evaluating the worm burden of *Mesocestoides corti* mice infection [50]. In contrast, we observed that nanosuspensions of PZQ presented higher influence in the parasite metabolism suggesting that the selection of a specific drug delivery system is a key factor. Thus, the design of PZQ nanosystems represents an attractive approach to treat NCC due to the increased availability of PZQ at the target site.

Conclusions

The present study evaluated the suitability of the nanoprecipitation process approach applied to the formulation of PZQ nanosuspensions. In agreement with these findings, the physicochemical characterization and solubility assay of the PZQ nanosuspensions confirmed that such technique and the chosen stabilizers were suitable to obtain the corresponding PZQ nanoformulations. Physical stability study at both 4 ± 2 and 25 ± 2 °C indicated that PZQ nanoparticles were stable in terms of solubility and particle size after 120-day storage. In vivo studies demonstrated that those nanosystems were able to produce significant modifications on the concentrations of oxaloacetate, citrate, pyruvate, alpha-ketoglutarate, malate, succinate, lactate, beta-hydroxybutyrate, fumarate, and propionate involved in the metabolism of *T. crassiceps* cysticerci. Therefore, these nanoformulations may be considered as a promising tool to deliver PZQ to the brain for the effective management of NCC.

Acknowledgments EA thanks CONICET (Argentina) for a fellowship.

Funding information This study received financial support from CNPq (Brazil) (grant no. 471009/2013-0), MINCYT (Argentina) (PICT 2012-1078), CONICET (Argentina), and National University of Rosario (Argentina).

Compliance with ethical standards

Animal studies All institutional and national guidelines for the care and use of laboratory animals were followed. This study was authorized by the Ethics Committee for Animal Use (CEUA/UFG) (registration number 050/13).

Conflict of interest The authors declare that they have no conflict of interest.

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