Formulation, characterization and pharmacokinetics of praziquantel-loaded hydrogenated castor oil solid lipid nanoparticles

Aim: The purpose of this study was to formulate praziquantel (PZQ)-loaded hydrogenated castor oil (HCO) solid lipid nanoparticles (SLN) to enhance the bioavailability and prolong the systemic circulation of the drug. **Materials & methods:** PZQ was encapsulated into HCO nanoparticles by a hot homogenization and ultrasonication method. The physicochemical characteristics of SLN were investigated by optical microscope, scanning electron microscopy and photon correlation spectroscopy. Pharmacokinetics were studied after oral, subcutaneous and intramuscular administration in mice. **Results:** The diameter, polydispersivity index, ζ potential, encapsulation efficiency and loading capacity of the nanoparticles were 344.0 ± 15.1 nm, 0.31 ± 0.08, -16.7 ± 0.5 mV, 62.17 ± 6.53% and 12.43 ± 1.31%, respectively. *In vitro* release of PZQ-loaded HCO-SLN exhibited an initial burst release followed by a sustained release. SLN increased the bioavailability of PZQ by 14.9-, 16.1- and 2.6-fold, and extended the mean residence time of the drug from 7.6, 6.6 and 8.2 to 95.9, 151.6 and 48.2 h after oral, subcutaneous and intramuscular administration, respectively. **Conclusion:** The PZQ-loaded HCO-SLN could be a promising formulation to enhance the pharmacological activity of PZQ.

KEYWORDS: bioavailability ■ pharmacokinetics ■ praziquantel ■ solid lipid nanoparticles ■ systemic circulation

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Schistosomiasis is a serious public health problem in tropical and subtropical regions, and is associated with a variety of clinical syndromes that may lead to severe morbidity. It is the second most common parasitic disease after malaria [1]. The disease is endemic in approximately 70 countries, and estimates for 2003 suggest that 207 million people are infected and 779 million are at risk [2]. Schistosomiasis afflicts populations living close to water (particularly lakes, ponds and irrigation canals) that is contaminated with the parasite – the same water that is also required for everyday activities such as cooking, washing and the generation of income through agriculture and/or fishing [2,3].

Praziquantel (PZQ) is a broad-spectrum antitrematode drug and has proven to be especially useful in the treatment of schistosomiasis [3,4]. The drug is effective against all important adult schistosome species [5,6]. Hence, PZQ has become the backbone of the global schistosomiasis control program in the disease endemic regions. PZQ also plays a role in transmission control of schistosomiasis to prevent acute infection [7]. However, owing to the fact that therapy is not sufficiently long lasting because of the fast metabolism of the drug [8,9], failures of mass treatment to control schistosomiasis have occurred [10–12]. Another factor that influences the effectiveness of the treatment is the low bioavailability of PZQ, due to its low hydrosolubility and the effect of first-pass metabolism [9,13]. After oral administration, PZQ is extensively converted into an inactive or considerably less potent compound [13,14].

Praziquantel is administered to humans only by the oral route [15]. Studies on PZQ pharmaceutics have been carried out to improve the bioavailability and systemic circulation of the drug for oral delivery. Administration of PZQ concomitant with cimetidine or food increases the levels of PZQ in the plasma, with an improvement in the treatment outcome [16–19]. Other studies have described the improvement of dissolution rate using adjuvant treatment, such as β-cyclodextrin and polyvinylpyrrolidone, to increase the pharmacological activity of PZQ [9,20,21]. Oral liposomal formulations improved both the bioavailability [22] and the antischistosomal activity of PZQ [23].

Studies demonstrated that PZQ was more effective by subcutaneous and intramuscular injection than oral administration [24,25]. The injectable formulation can be given either subcutaneously or intramuscularly in veterinary medicine [26]. If alternative routes of delivery in humans, such as parenteral formulations, could be considered, drugs administered by such routes would achieve direct systemic delivery,

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thereby avoiding first-pass hepatic metabolism, improving therapy efficacy and reaching a reduction in the dose delivered [15].

In the past decades, solid lipid nanoparticles (SLN) have received considerable attention in the field of drug delivery as an alternative dosage form to other colloid nanoparticles [27]. The main advantages of SLN are that they have good biocompatibility and biodegradability, high bioavailability, offer sustained release, can be produced on a large scale and can be delivered by almost all routes [28–31]. Our previous work demonstrated that hydrogenated castor oil (HCO)-SLN were an effective nanoparticle system for controlled release and enhancement of pharmacological activities of encapsulated drugs [28,29,32]. In this work, PZQ-loaded HCO-SLN were formulated and administered to mice by different routes. The pharmacokinetics of the formulation was studied to evaluate the improvement of the bioavailability and the systemic circulation of PZQ.

Materials & methods

Materials

Hydrogenated castor oil was purchased from Tongliao Tonghua Castor Chemical Co., Ltd. (Inner Mongolia, China). PZQ reference standard was bought from the China Institute of Veterinary Drug Control (Beijing, China). PZQ was obtained from Wuhan Kanglong Century Technology Development Co., Ltd (Wuhan, China). Poly vinyl alcohol (PVA) was purchased from Sigma (MO, USA). Methyl alcohol used for high performance liquid chromatography (HPLC) was of liquid chromatography grade, available from Tedia Company, Inc. (USA). The water for HPLC was prepared with a Milli-Q system (Millipore, MA, USA).

Preparation of PZQ-loaded HCO-SLN

Solid lipid nanoparticles were prepared by hot homogenization and ultrasonication method. HCO (1.6 g) and PZQ (0.4 g) were added to a 50 ml tube and put in a boiling water bath. After the lipid was melted and the drug was dissolved in the melted lipid, 40 ml 1% PVA solution preheated in a boiling water bath was poured into the lipid phase under magnetic stirring to form an oil–water emulsion, and then sonicated for 5 min (VC X 750 Vibra-Cell™, Sonics and Materials, Inc., Newtown, CT, USA, using the 13 mm microprobe with amplitude 35%) to form a nanoemulsion. The hot nanoemulsion was quickly poured into 200 ml cold water to obtain a nanoparticle suspension. The nanoparticles were collected by centrifugation at 12,000 rpm (Centrifuge 5810 R; Eppendorf, Germany) for 90 min at 4°C, and washed three times with distilled water. The SLN were suspended in 30 ml distilled water and lyophilized for 48 h (LGJ-12 Freeze Dryer; Beijing Songyuanhuaxing Science Technology Development Co., Ltd., China). The control nanoparticles were prepared in the same way without adding the PZQ.

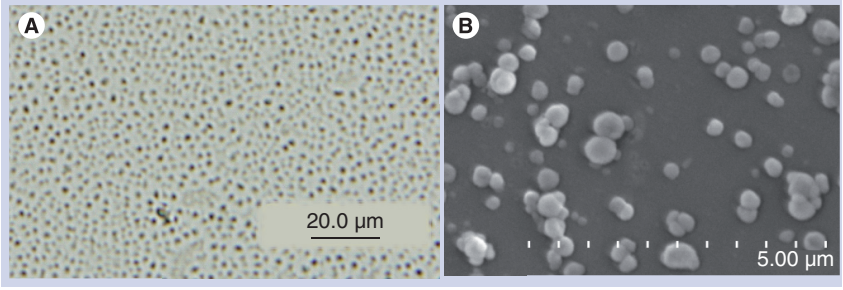

Figure 1. Photographs of (A) optical microscope (magnification ×400) and (B) scanning electron microscope of praziquantel-loaded hydrogenated castor oil solid lipid nanoparticles.

Microscopic analysis

A total of 1 mg of PZQ-SLN was suspended in 1 ml ethanol. 2 µl of the suspension were placed on a microscope slide and the nanoparticles were dried at room temperature for 5 min. Photomicrographs of the SLN were taken using an inverted optical microscope (Olympus 1X71, Olympus, Japan).

Scanning electron microscopy

The morphology of PZQ-SLN was studied by scanning electron microscopy (SE S-3400N; Hitachi, Japan). Briefly, 1 mg samples were suspended in 1 ml distilled water and 2 µl of the suspension were placed on a glass slide. After oven drying at 45°C for 10 min, the samples were coated with gold using an Ion Sputter and examined at an accelerating voltage of 20 kV.

Determination of particle size, polydispersity index & ζ potential

The particle size, polydispersity index (PDI) and ζ potential analysis of PZQ-SLN was performed by photon correlation spectroscopy (PCS) using Zetasizer Nano ZS90 (Malvern Instruments, UK). The samples were suspended in distilled water at a concentration of 2.7 mg/ml for particle size and PDI determination, and a concentration of 0.3 mg/ml for ζ potential determination.

Determination of loading capacity & encapsulation efficiency

To determine the drug content in the nanoparticles, a weighed amount of freeze-dried PZQ-SLN was suspended in methyl alcohol

Table 1. Characteristics of praziquantel-loaded hydrogenated castor oil solid lipid nanoparticles.

EE (%)	LC (%)	MD (nm)	PDI	ZP (mv)	RP (%)
62.17 ± 6.53	12.43 ± 1.31	344.0 ± 15.1	0.31 ± 0.08	-16.7 ± 0.5	0.53 ± 0.11

Mean ± standard deviation; n = 3.
EE: Encapsulation efficiency; LC: Loading capacity; MD: Mean diameter; PDI: Polydispersity index; RP: Residual polyvinyl alcohol; ZP: ζ potential.

in a 15 ml tube and was heated on a boiling water bath for 10 min. After cooling down for 20 min at room temperature, the solution was centrifuged at 9000 rpm (Centrifuge 5810 R; Eppendorf, Germany) for 15 min and the supernatant was taken for PZQ quantitation by HPLC using a UV detector at wavelength 215 nm (Shimadiu Corporation, Kyoto, Japan). The control nanoparticles formulated without PZQ were treated similarly and used as blanks for the measurements. The assay was repeated three times using different samples from independent preparations. Loading capacity and encapsulation efficiency are defined as follows:

$$Loading\ capacity = \left(\frac{Weight\ of\ PZQ\ in\ SLN}{Weight\ of\ SLN}\right) x\,100$$

$$Encapsulation\ efficiency = \left(\frac{Weight\ of\ PZQ\ in\ SLN}{Weight\ of\ PZQ\ added}\right) x100$$

Determination of residual PVA

The amount of PVA associated with HCO-SLN was determined by a colorimetric method described previously [26]. Briefly, 8 mg of lyophilized nanoparticle sample was treated with 2 ml 0.5 M NaOH for 15 min at 60°C. Each sample was neutralized with 900 µl 1 M HCl and the volume was adjusted to 5 ml with distilled water. The solution was filtered with 0.22 µm filters and mixed with 3 ml 0.65 M boric acid solution, 0.5 ml iodine/potassium iodide (0.05 M/0.15 M) solution and 1.5 ml distilled water. Following incubation at room temperature for 15 min, the absorbance was measured at 690 nm using a UV spectrophotometer (U-800, Hitachi, Japan). A standard plot of PVA was completed under identical conditions. Residual PVA (RP) is defined as follow:

$$RP = \left(\frac{Amount\ of\ PVA\ associated\ with\ SLN}{Weight\ of\ SLN}\right) x\,100$$

In vitro release studies

Praziquantel-loaded HCO-SLN (10 mg) were suspended in 2 ml of 0.9% (w/v) NaCl solution (donor solution) in a dialysis bag (molecular weight: 8000–14,400) and dialyzed against 45 ml NaCl solution (0.9%, w/v, receiver solution) in a 50 ml tube at 37°C under magnetic stirring at 100 rpm. To determine the PZQ amount diffused through the dialysis bag, samples (1 ml) were taken from the receiver solution and the same amount of fresh medium was added to keep a constant volume at fixed time points. PZQ in the samples was measured by HPLC. The control nanoparticles formulated without PZQ were treated similarly and used as blanks for the measurements.

In vivo study

Kunming species mice (male, 30 ± 1 g) were obtained from the Medical Animal Test Center of Peking University, China. All experimental protocols concerning the handling of mice were in accordance with the requirements of the Institutional Animal Care and Use Committee at China Agricultural University. The animals were housed at room temperature with free access to a standard diet and water for 1 week before use. Before initiation of the experiment, 96 mice were randomly divided into 24 groups with four animals in each group. Then, 0.9 mg PZQ dissolved in 50 µl 25% alcohol solution or 7.24 mg PZQ-SLN (containing 0.9 mg PZQ) suspended in 100 µl sterile distilled water was

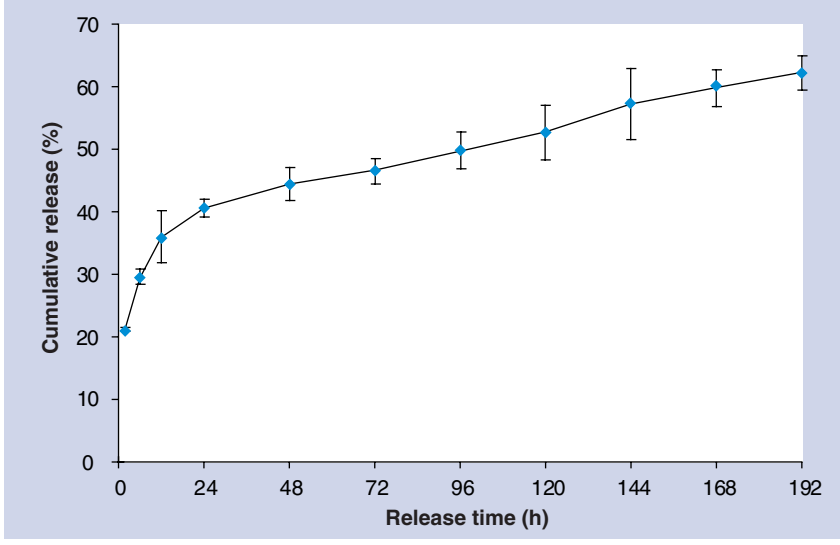

Figure 2. *In vitro* release of praziquantel-loaded hydrogenated castor oil solid lipid nanoparticles (mean ± standard deviation; n = 3).

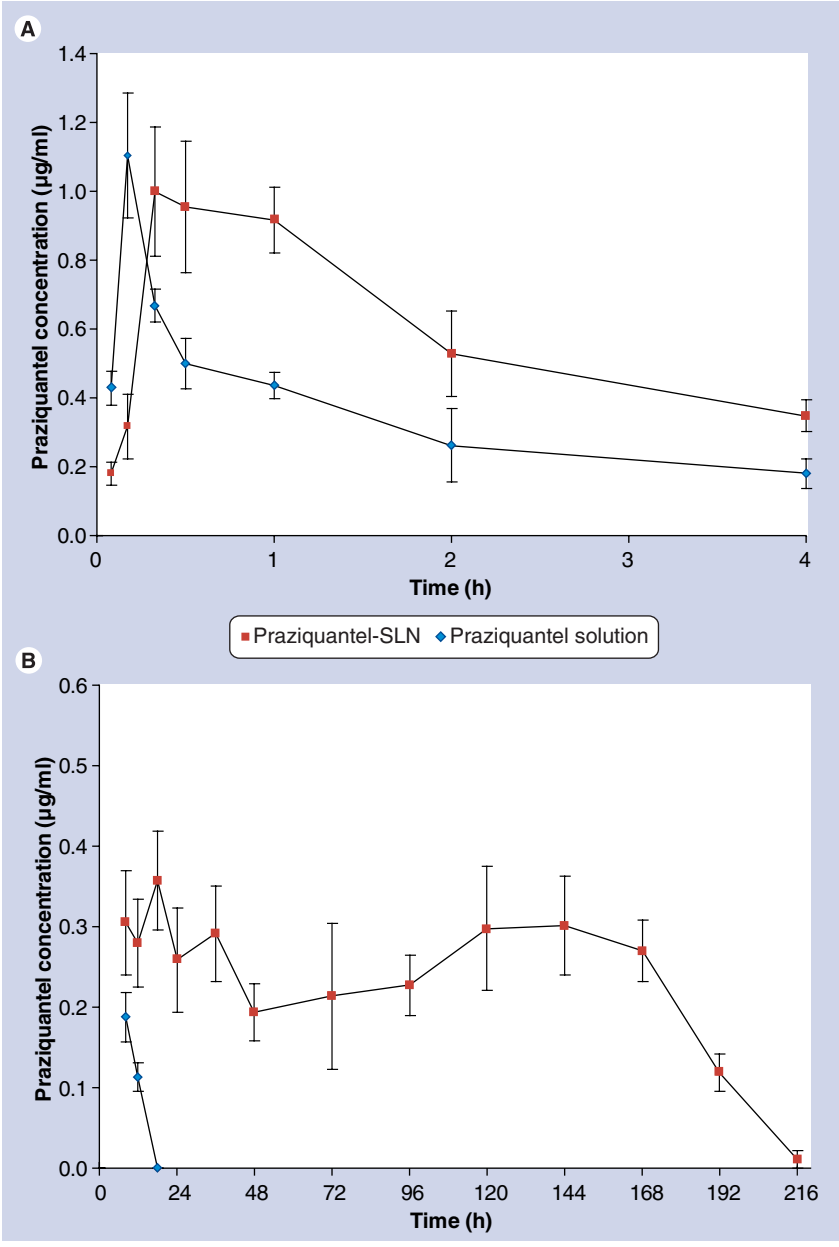

Figure 3. Plasma praziquantel concentration–time curves after a single dose of oral administration of praziquantel-solid lipid nanoparticles and praziquantel solution (30 mg/kg; mean ± standard deviation; n = 4) (A) within 4 h; (B) from 8 to 192 h.
SLN: Solid lipid nanoparticle.

administered to each mouse (30 mg/kg) in the different groups by oral, intramuscular or subcutaneous routes, respectively. Each route contained four groups for SLN treatment and four groups for PZQ solution treatment. At specific time points (0.08, 0.17, 0.33, 0.5, 1, 2, 4, 8, 12, 18, 24, 36, 48, 72, 96, 120, 144, 168, 192, 216, 240, 268 and 292 h) after administration, blood samples were taken from a tail vein and the drug levels in the plasma were assayed. For each treatment, the blood samples were collected by turns from four groups over the observation period owing to the limited blood volume that could be safely collected from each mouse.

HPLC assay

Praziquantel concentrations in plasma were measured by HPLC. Chromatographic conditions were as follows: VP-ODS: 250 × 4.6 mm (Shimadiu Coperation, Kyoto, Japan); mobile phase: methyl alcohol/Milli-Q water (65/35, v/v); flow rate: 1.0 ml/min; UV detector wavelength: 215 nm. Sample extracts were prepared by mixing 100 µl plasma with 200 µl mixture of methyl alcohol and acetonitrile (1/1, v/v). The mixtures were vortexed for 3 min to allow complete mixing, followed by centrifugation at 14,000 rpm (Sigma 1–14; Sartorius, Germany) for 25 min. Then, 50 µl supernatant was taken for HPLC analysis. The plasma PZQ concentration was found to be linear over the range of 0.05 to 8 µg/ml. The correlation coefficient was 0.9997. The relative standard deviations of accuracy and precision for three different plasma PZQ concentrations (0.1, 1 and 4 µg/ml) were 15.4, 4.23 and 0.56%, respectively, for interday analysis, and 6.67, 3.80 and 5.46%, respectively, for intraday analysis.

Pharmacokinetic analysis

The plasma PZQ concentrations versus time data were analyzed based on noncompartmental pharmacokinetics using the PK Solutions 2.0 computer software (Ashland, OH, USA).

Statistical methods

The loading capacity, encapsulation efficiency, PDI, ζ potential, residual PVA and pharmacokinetic parameters data were analyzed using one-way ANOVA. Significance was evaluated at a p-value of 0.05. Statistical analysis was performed using Microsoft Excel (2003).

Results

Physicochemical characteristics of SLN

Photomicrographs showed that SLN were well dispersed with good particle size distributions (Figure 1A). Scanning electron microscopy studies demonstrated that the nanoparticles were spherical and had smooth surfaces (Figure 1B). Further detailed PCS analysis indicated the average particle size (hydrodynamic diameter) of SLN was 344.0 ± 15.1 nm with polydispersivity index 0.31 ± 0.08 and ζ potential -16.7 ± 0.5 mV (Table 1). PZQ was encapsulated efficiently in nanoparticles. The mean entrapment efficiency

was 62.17 ± 6.53% and the average loading of PZQ in nanoparticles was 12.43 ± 1.31%. The residual PVA associated with the SLN was 0.53 ± 0.11% (Table 1).

In vitro release

In vitro release of PZQ from the nanoparticles is illustrated in Figure 2. The SLN exhibited a burst release effect with approximately 21.27% of the drug released within the initial 2 h. The release reached 40.69% in 24 h and then the nanoparticles displayed a sustained release phase. The amount of cumulative drug released over 7 days was approximately 62.24%.

Pharmacokinetics

Following oral administration, the trend of plasma PZQ concentration–time curves of PZQ-SLN and PZQ solution was similar, but the increase and decrease of the drug in nanoparticle groups was significantly slower. The plasma drug concentration for PZQ-SLN increased to a maximum concentration of approximately 1.1 μg/ml at 0.44 h. The drug concentration decreased slowly and was sustained above the minimal effective concentration (0.1 μg/ml [33]) for 192 h (Figure 3). For the PZQ solution, the plasma drug concentration increased rapidly to a peak level of 1.1 μg/ml at 0.17 h, then declined quickly and was not detectable at 18 h postadministration (Figure 3). The pharmacokinetic analysis revealed that the time to maximum drug concentration (T_{max}), half-life of absorption ($T_{1/2ab}$), half-life of elimination ($T_{1/2el}$) and mean residence time (MRT) of PZQ-SLN were significantly longer than those of PZQ solution (Table 2). The bioavailability of PZQ-SLN was also increased significantly compared with that of PZQ solution.

The drug concentration after subcutaneous injection of PZQ-SLN increased to 1.4 μg/ml within 0.33 h, then declined slowly and was sustained over 0.1 μg/ml (Figure 4) for 264 h. By contrast, the drug concentration for PZQ solution groups increased swiftly to 4.5 μg/ml at 0.33 h, and then sharply decreased and was below 0.1 μg/ml at 12 h (Figure 4). The bioavailability of PZQ-SLN was significantly higher than that of PZQ solution (101.6 vs 6.3 mg h/l). The MRT, $T_{1/2ab}$ and $T_{1/2el}$ of PZQ-loaded SLN (151.6, 8.2 and 29.6 h, respectively) were much longer than those obtained with PZQ solution (6.6, 1.5 and 2.3 h, respectively) (Table 2).

In the intramuscular administration groups, the plasma drug level of PZQ-SLN reached a peak of 2.7 μg/ml at 0.33 h and this was maintained over 0.1 μg/ml for 72 h (Figure 5). In the case of PZQ solution, the drug concentration swiftly increased to a peak of 5.4 μg/ml at 0.17 h, subsequently exhibited a precipitous drop and decreased below 0.1 μg/ml 12 h postinjection (Figure 5). Pharmacokinetic data indicated that the SLN enhanced the bioavailability of PZQ 2.6-fold and extended the MRT from 8.2 to 48.2 h (Table 2). Compared with oral and subcutaneous administration, intramuscular injection of PZQ-SLN had fewer effects for the improvement of systemic circulation and bioavailability of the drug.

Discussion

Praziquantel is practically insoluble in water and has poor bioavailability owing to extensive hepatic first-pass metabolism [3] and rapid clearance from the bloodstream, with a terminal $T_{½el}$ of 1–3 h [34]. Therefore, new delivery strategies need to be developed to improve its bioavailability and systemic circulation. In this study, PZQ-loaded HCO-SLN was prepared by a hot homogenization and ultrasonication method. This preparation method avoided using organic solvent, while at the same time maintaining drug stability – the temperature in the preparation did

Table 2. Pharmacokinetic parameters of praziquantel-solid lipid nanoparticles and praziquantel solution after oral, subcutaneous and intramuscular administration in mice.

Administration routes	Dose	MRT (h)	$AUC_{0-\infty}$ (mg.h/l)	C_{max} (μg/ml)	T_{max} (h)	$T_{½ab}$ (h)	$T_{½el}$ (h)
Oral	Solution	7.6 ± 0.9	4.1 ± 1.1	1.1 ± 0.2	0.17	2.0 ± 1.5	4.7 ± 1.2
	SLN	95.9 ± 4.3†	61.0 ± 2.7†	1.1 ± 0.1	0.44 ± 0.10†	15.3 ± 4.2†	20.1 ± 1.3†
Subcutaneous	Solution	6.6 ± 1.9	6.3 ± 0.4	4.5 ± 0.5	0.33	1.5 ± 0.9	2.3 ± 0.4
	SLN	151.6 ± 9.3†	101.6 ± 9.2†	1.4 ± 0.1†	0.33	8.2 ± 0.9†	29.6 ± 1.4†
Intramuscular	Solution	8.2 ± 0.7	10.1 ± 0.4	5.4 ± 0.3	0.17	0.16 ± 0.04	4.3 ± 1.5
	SLN	48.2 ± 6.8†	26.2 ± 1.3†	2.7 ± 0.2†	0.33 ± 0.17	0.75 ± 0.13†	11.7 ± 5.4†

Mean ± standard deviation; n = 4.
†Statistical significances between praziquantel-SLN and praziquantel solution are $p < 0.05$.
$AUC_{0-\infty}$: Area under the concentration–time curve from zero to infinity; C_{max}: Maximal praziquantel concentration in plasma; MRT: Mean residence time; SLN: Solid lipid nanoparticles; $T_{½ab}$: Absorption half-life; $T_{½el}$: Elimination half-life; T_{max}: Time to reach C_{max}.

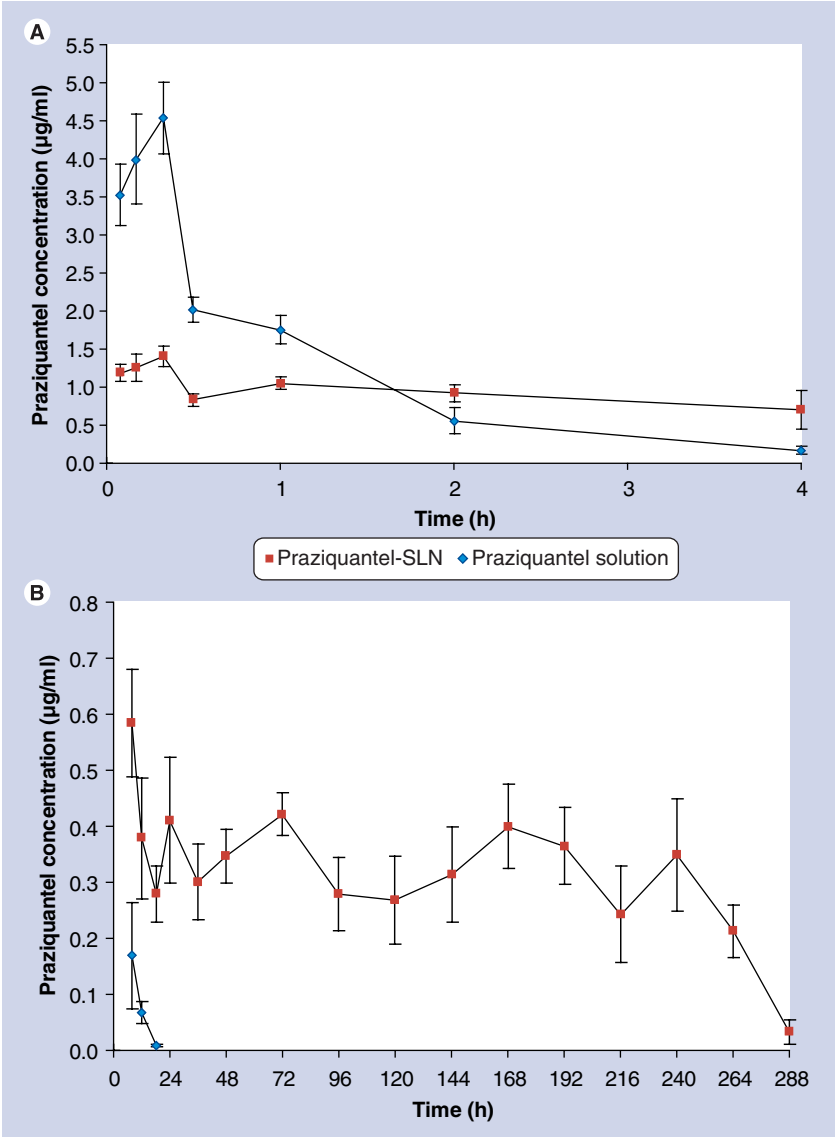

Figure 4. Plasma praziquantel concentration–time curves after a single dose of subcutaneous administration of praziquantel-solid lipid nanoparticles and praziquantel solution (30 mg/kg; mean ± standard deviation; n = 4) (A) within 4 h and (B) from 8 to 288 h.
SLN: Solid lipid nanoparticle.

not exceed 100°C. The melting point of PZQ is within the range of 136 to 140°C [34]. The preparation resulted in consistent nanoparticles of 344.0 ± 15.1 nm with narrow size distribution. These results demonstrated that the hot homogenization and ultrasonication method was a feasible method for preparation of PZQ-loaded HCO-SLN. For SLN formulation, solubility of drug in the lipid determines the encapsulation efficiency of lipid nanoparticles. As PZQ has good liposolubility, it was encapsulated efficiently in the HCO-SLN. PVA is the most commonly used emulsifier in the formulation of nanoparticles due to its excellent mechanical strength, biocompatibility and nontoxicity, and has been approved by the US FDA for medical and food applications [35]. In this formulation, the residual PVA associated with the nanoparticles was lower compared with other studies [36,37].

The *in vitro* release profile demonstrated that the release pattern of PZQ-loaded HCO-SLN is very similar to that of other drug-loaded HCO nanoparticles prepared by an organic solvent evaporation method [27,28,32,38], suggesting that the release character of HCO-SLN mainly depends on the lipid matrix rather than the drug and preparation method.

The *in vivo* study demonstrated that the PZQ-loaded HCO-SLN, delivered by oral, subcutaneous and intramuscular administration, improved the bioavailability and extended systemic circulation of PZQ to a large extent. The enhanced absorption and extended residence time of PZQ could be attributed to a number of reasons. After oral administration, owing to their small particle size, SLN may exhibit bioadhesion to the GI tract wall or enter the intervillar spaces, thus increasing their residence time in the GI tract [31]. The small size of SLN also contributes to an increase in the permeability of the intestinal membrane [39]. The large surface area of nanoparticles can increase the dissolution rate of highly insoluble drugs [31]. The increased adhesion and permeability, and improved dissolution rate will result in enhanced bioavailability [31,39]. The higher bioavailability could also be due to an enhanced lymphatic uptake [40,41]. The enhanced lymphatic transport of the drug can reduce the hepatic first pass metabolism and improve bioavailability of PZQ because the intestinal lymph vessels drain directly into the thoracic duct, further into the venous blood, thus bypassing the portal circulation [42,43]. The increased residence time of the nanoparticles in the GI tract could extend the $T_{1/2ab}$ and $T_{1/2el}$ of PZQ-SLN, leading to longer MRT of the drug. When PZQ was injected as nanoparticle formulations, the distribution of PZQ was considered to be limited because the nanoparticles formed a sustained release depot at the injection sites [44]. After injection, nanoparticles do not have direct access to the bloodstream. Instead, they are taken up by regional lymph nodes or remain at the site of injection. When injected as solution, PZQ would spread widely from the injection sites. The delayed local dissolution and transport through cellular interstitia into blood circulation can result in a significantly prolonged circulation effect [45]. Furthermore, SLN can protect the drug from chemical and enzymatic degradation, thereby delaying the *in vivo* metabolism [32]. In addition, SLN gradually release entrapped PZQ

from the lipid matrix into blood, and thus extend the duration of therapeutic concentration in the circulation [46].

Subcutaneous delivery of the PZQ-SLN produced the longest therapeutic concentration in the circulation and the highest bioavailability compared with oral and intramuscular administration. Subcutaneous injection results in delivery of SLN to the interstitium area underlying the dermis of the skin [45,47]. Passage through the interstitium to the vascular or lymphatic capillaries can also present a barrier to efficient drug absorption after subcutaneous administration, and thus lead to the delayed rate of absorption [48]. Intramuscular administration of PZQ-SLN produced the highest plasma levels of PZQ and shortest duration on the systemic circulation, which was consistent with other research [49].

The schistosomicidal effect of PZQ does not depend on the maximum drug concentration to which schistosomes are exposed, but rather on the length of time during which parasites are exposed to a threshold drug concentration [16]. The work of Gonnert *et al.* demonstrated that splitting of the total dose into three or more fractional doses, given within 1 day, approximately doubles the efficacy over that achieved after a single oral administration of the same total dose [24]. A higher therapeutic efficacy was obtained by repeated administration on each consecutive day than a single administration with a higher dose [7]. The sustained concentration of PZQ at an early stage of infection can kill the majority or even all of the female worms just after the worms have reached maturity and commenced egg production [50]. The results presented in this article suggest that PZQ-SLN could be a promising formulation to improve the schistosomicidal effect of PZQ, but further studies need to be carried out to demonstrate its prophylactic and therapeutic efficacy.

In spite of the reduced effectiveness in improving the bioavailability and systemic circulation compared with injection routes, oral administration of PZQ-loaded HCO-SLN could be the first choice owing to the noninvasive nature and convenience – patient compliance is a major concern when developing a drug-delivery system [41].

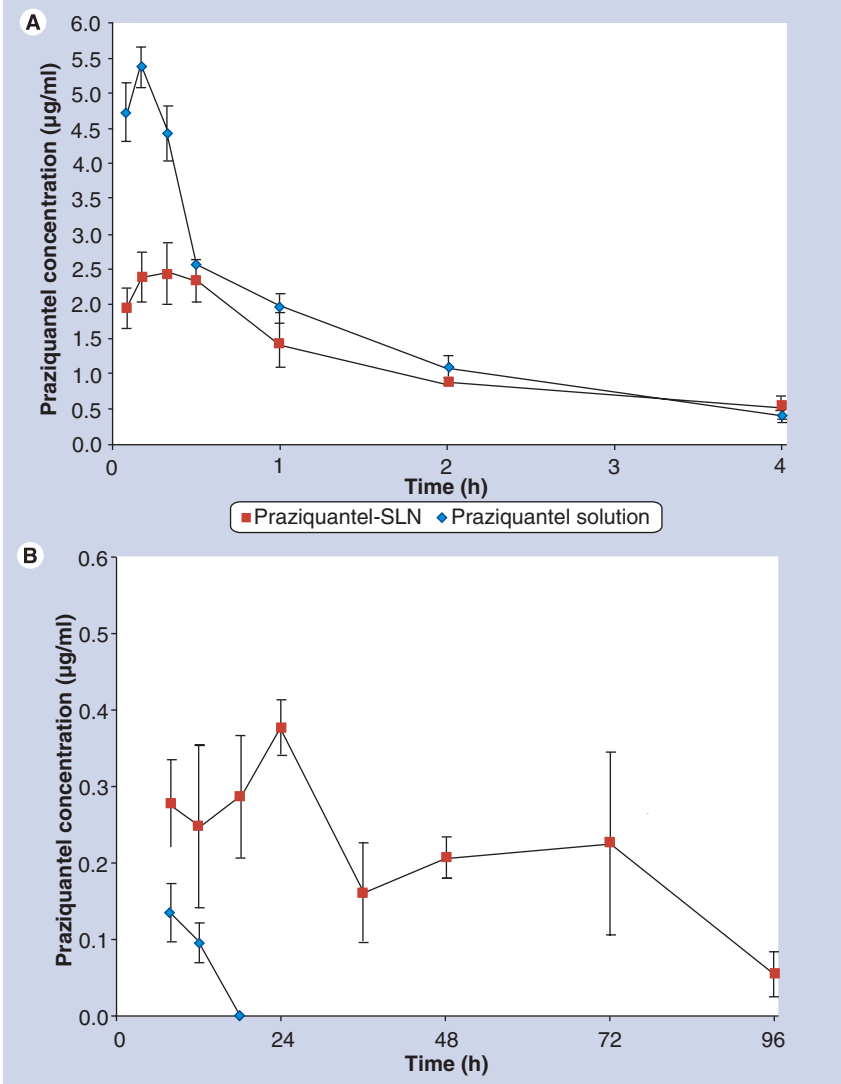

Figure 5. Plasma praziquantel concentration–time curves after a single dose of intramuscular administration of praziquantel-solid lipid nanoparticles and praziquantel solution (30 mg/kg) (mean ± standard deviation; n = 4) (A) within 4 h and (B) from 8 to 96 h.
SLN: Solid lipid nanoparticle.

Parenteral administration of PZQ-SLN could be a candidate route for improving the therapy efficacy and reducing the dose delivered.

Conclusion

A hot homogenization and ultrasonication method has been used for preparation of PZQ-loaded HCO-SLN. HCO-SLN improve bioavailability and extends the systemic circulation

Executive summary

- The hot homogenization and ultrasonication method was feasible for producing praziquantel-loaded hydrogenated castor oil solid lipid nanoparticles (PZQ-loaded HCO-SLN).
- The PZQ-loaded HCO-SLN had a sustained-release effect.
- The PZQ-loaded HCO-SLN significantly improved the bioavailability and extended the systemic circulation of PZQ by oral, subcutaneous and intramuscular administration; subcutaneous administration showed the best improvement.
- HCO-SLN could be a promising delivery system to improve the pharmacological activity of PZQ.

time of PZQ significantly by subcutaneous, oral and intramuscular administration. The nanoparticle system could be an effective vehicle for delivery of PZQ for enhanced therapy and control of schistosomiasis infection.

Future perspective

Future studies will be focused on the toxicity, prophylactic and therapeutic effects of PZQ-loaded HCO-SLN.

Financial & competing interests disclosure

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

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Bibliography

1 Chitsulo L, Engels D, Montresor A, Savioli L: The global status of schistosomiasis and its control. *Acta Trop*. 77, 41–51 (2000).

2 Steinmann P, Keiser J, Bos R, Tanner M, Utzinger J: Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. *Lancet Infect. Dis*. 6, 411–425 (2006).

3 Caffrey CR: Chemotherapy of schistosomiasis: present and future. *Curr. Opin. Chem. Biol*. 11, 433–439 (2007).

4 Meier H, Blaschke G: Investigation of praziquantel metabolism in isolated rat hepatocytes. *J. Pharm. Biomed. Anal*. 26, 409–415 (2001).

5 Harder A: Chemotherapeutic approaches to schistosomes: current knowledge and outlook. *Parasitol. Res*. 88, 395–397 (2002).

6 Zhou XN, Chen MG, Mcmanus D, Bergquist R: Schistosomiasis control in the 21st century: proceedings of the International Symposium on Schistosomiasis, Shanghai, July 4–6, 2001. *Acta Trop*. 82, 95–114 (2002).

7 Chen MG: Use of praziquantel for clinical treatment and morbidity control of schistosomiasis japonica in China: a review of 30 years' experience. *Acta Trop*. 96, 168–176 (2005).

8 Akbarieh M, Besner JG, Galal A, Tawafhail R: Liposomal system for the targeting and controlled release of praziquantel. *Drug Dev. Ind. Pharm*. 18, 303–307 (1992).

9 El-Arini, SK, Leuenberger H: Dissolution properties of praziquantel – PVP systems. *Pharm. Acta Helv*. 73, 89–94 (1998).

10 Danso-Appiah A, De Vlas SJ: Interpreting low praziquantel cure rates of *Schistosoma mansoni* infections in Senegal. *Trends Parasitol*. 18, 125–129 (2002).

11 Doenhoff MJ, Kusel JR, Coles GC, Cioli D: Resistance of *Schistosoma mansoni* to praziquantel: is there a problem? *Trans. R. Soc. Trop. Med. Hyg*. 96, 465–469 (2002).

12 Cioli D: Praziquantel: is there real resistance and are there alternatives? *Curr. Opin. Infect. Dis*. 13, 659–663 (2000).

13 Xiao SH, Catto BA, Webster LT: Effects of praziquantel on different developmental stages of *Schistosoma mansoni in vitro* and *in vivo*. *J. Infect. Dis*. 151, 1130–1137 (1985).

14 Andrews PA: Summary of the efficacy of praziquantel against schistosomes in animal experiments and notes on its mode of action. *Arzneimittelforschung* 31(3a), 538–541 (1981).

15 Mainardes RM, Evangelista RC: PLGA nanoparticles containing praziquantel: effect of formulation variables on size distribution. *Int. J. Pharm*. 290, 137–144 (2005).

16 Jung H, Medina R, Castro N, Corona T, Sotelo J: Pharmacokinetic study of praziquantel administered alone and in combination with cimetidine in a single-day therapeutic regimen. *Antimicrob. Agents Chemother*. 41, 1256–1259 (1997).

17 Castro N, Jung H, Medina R, Gonzalez-Esquivel D, Lopez M, Sotelo J: Interaction between grapefruit juice and praziquantel in humans. *Antimicrob. Agents Chemother*. 46, 1614–1616 (2002).

18 Metwally AA, Bennett JL, Botros SS, Ebeid FA: Effect of cimetidine, bicarbonate and glucose on the bioavailability of different formulations of praziquantel. *Drug Res*. 45(4), 516–518 (1995).

19 Castro N, Medina R, Sotelo J, Jung H: Bioavailability of praziquantel increases with concomitant administration of food. *Antimicrob. Agents Chemother*. 44, 2903–2904 (2000).

20 Becket G, Schep LJ, Tan MY: Improvement of the *in vitro* dissolution of praziquantel by complexation with α-, β- and γ-cyclodextrins. *Int. J. Pharm*. 179, 65–71 (1999).

21 De Jesus MB, De Matos Alves Pinto L, Fraceto LF *et al.*: Theoretical and experimental study of a praziquantel and β-cyclodextrin inclusion complex using molecular mechanic calculations and H1-nuclear magnetic resonance. *J. Pharm. Biomed. Anal*. 41, 1428–1432 (2006).

22 Bayomi MA, Al-Suwayeh SA, El-Helw AM, Mesnad AF: Preparation of casein-chitosan microspheres containing diltiazem hydrochloride by an aqueous coacervation technique. *Pharm. Acta Helv*. 73, 187–192 (1998).

23 Mourao SC, Costa PI, Salgado HR, Gremiao MP: Improvement of antischistosomal activity of praziquantel by incorporation into phosphatidylcholine-containing liposomes. *Int. J. Pharm*. 295, 157–162 (2005).

24 Gonnert R, Andrews P: Praziquantel, a new board-spectrum antischistosomal agent. *Z. Parasitenkd*. 52, 129–150 (1977).

25 Webbe G, James C: A comparison of the susceptibility to praziquantel of *Schistosoma haematobium, S. japonicum, S. mansoni, S. intercalatum* and *S. mattheei* in hamsters. *Z. Parasitenkd*. 52, 169–177 (1977).

26 Dayan AD: Albendazole, mebendazole and praziquantel. Review of non-clinical toxicity and pharmacokinetics. *Acta Trop*. 86, 141–159 (2003).

27 Xie SY, Wang SL, Zhao BK *et al.*: Effect of PLGA as a polymeric emulsifier on preparation of hydrophilic protein-loaded solid lipid nanoparticles. *Colloids Surf. B Biointerfaces* 67, 199–204 (2008).

28 Han C, Qi CM, Zhao BK *et al.*: Hydrogenated castor oil nanoparticles as carriers for the subcutaneous administration of tilmicosin: *in vitro* and *in vivo* studies. *J. Vet. Pharmacol. Ther*. 32, 116–123 (2009).

29 Wang SL, Xie SY, Zhu LY, Wang FH, Zhou WZ: Effects of poly (lactic-co-glycolic acid) as a co-emulsifier on the preparation and hypoglycaemic activity of insulin-loaded solid lipid nanoparticles. *IET Nanobiotechnol*. 4, 103–108 (2009).

30 Duchêne D, Ponchel G: Bioadhesion of solid oral dosage forms, why and how? *Eur. J. Pharm. Biopharm.* 44(1),15–23 (1997).

31 Luo Y, Chen D, Ren L, Zhao X, Qin J: Solid lipid nanoparticles for enhancing vinpocetine's oral bioavailability. *J. Control. Release* 114, 53–59 (2006).

32 Li SY, Zhao BK, Wang FH *et al.*: Yak interferon-α loaded solid lipid nanoparticles for controlled release. *Res. Vet. Sci.* 88(1), 148–153 (2009).

33 Pica-Mattoccia L, Cioli D: Sex- and stage-related sensitivity of *Schistosoma mansoni* to *in vivo* and *in vitro* praziquantel treatment. *Int. J. Parasitol*. 34, 527–533 (2004).

34 Cioli D, Pica-Mattoccia L: Praziquantel. *Parasitol. Res*. 90(Suppl. 1), S3–S9 (2003).

35 DeMerlis CC, Schoneker DR: Review of the oral toxicity of polyvinyl alcohol (PVA). *Food Chem. Toxicol*. 41, 319–326 (2003).

36 Sahoo SK, Panyam J, Prabha S, Labhasetwar V: Residual polyvinyl alcohol associated with poly (D,L-lactide-co-glycolide) nanoparticles affects their physical properties and cellular uptake. *J. Control. Release* 82, 105–114 (2002).

37 Zambaux MF, Bonneaux F, Ref RG *et al.*: Influence of experimental parameters on the characteristics of poly(lactic acid) nanoparticles prepared by a double emulsion method. *J. Control. Release* 50, 31–40 (1998).

38 Xie SY, Wang SL, Zhu LY, Wang FH, Zhou WZ: The effect of glycolic acid monomer ratio on the emulsifying activity of PLGA in preparation of protein-loaded SLN. *Colloids Surf. B Biointerfaces* 74, 358–361 (2009).

39 Hu L, Tang X, Cui F: Solid lipid nanoparticles (SLNs) to improve oral bioavailability of poorly soluble drugs. *J. Pharm. Pharmacol*. 56, 1527–1535 (2004).

40 Holm R, Mullertz A, Christensen E, Hoy CE, Kristensen HG: Comparison of total oral bioavailability and the lymphatic transport of halofantrine from three different unsaturated triglycerides in lymph-cannulated conscious rats. *Eur. J. Pharm. Sci*. 14, 331–337 (2001).

41 Rieux AD, Fievez V, Garinot M, Schneider YJ, Préat V: Nanoparticles as potential oral delivery systems of proteins and vaccines: a mechanistic approach. *J. Control. Release* 116, 1–27 (2006).

42 Hussain N, Jaitley V, Florence AT: Recent advances in the understanding of uptake of microparticulates across the gastrointestinal lymphatics. *Adv. Drug Deliv. Rev.* 50, 107–142 (2001).

43 Wasan KM: Formulation and physiological and biopharmaceutical issues in the development of oral lipid-based drug delivery systems. *Drug Dev. Ind. Pharm*. 27, 267–276 (2001).

44 Barratt G: Colloidal drug carriers: achievements and perspectives. *Cell Mol. Life Sci*. 60, 21–37 (2003).

45 Kipp JE: The role of solid nanoparticle technology in the parenteral delivery of poorly water-soluble drugs. *Int. J. Pharm*. 284, 109–122 (2004).

46 Muller, RH, Mader K, Gohla S: Solid lipid nanoparticles (SLN) for controlled drug delivery – a review of the state of the art. *Eur. J. Pharm. Biopharm*. 50, 161–177 (2000).

47 Wissing SA, Kayser O, Muller RH: Solid lipid nanoparticles for parenteral drug delivery. *Adv. Drug Deliv. Rev.* 56, 1257–1272 (2004).

48 McLennan DN, Porter CJ, Charman SA: Subcutaneous drug delivery and the role of the lymphatics. *Drug Discov. Today Technol.* 2(1), 89–96 (2005).

49 Baert L, Klooster GV, Dries W *et al.*: Development of a long-acting injectable formulation with nanoparticles of rilpivirine (TMC278) for HIV treatment. *Eur. J. Pharm. Biopharm*. 72 (3), 502–508 (2009).

50 Xiao SH, You JQ, Mei JY, Jiao PY: Early treatment of schistosomal infection with praziquantel in mice. *Acta Trop*. 14, 533–538 (1993).