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Enhancement the oral bioavailability of praziquantel by incorporation into solid lipid nanoparticles

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The aim of this study was to assess the feasibility of solid lipid nanoparticles (SLN) to enhance the oral bioavailability of praziquantel (PZQ). SLN loaded with PZQ were produced by ultrasound technique. The characteristics of PZQ-SLN were studied in detail. The concentration of PZQ in plasma was determined using reversed-phase high-performance liquid chromatography after oral administration of PZQ-SLN and control PZQ tablets (PZQ-TAB) in rats respectively. The results showed that PZQ-SLN had an average diameter 110 nm with Zeta potential of −66.3 mV. The encapsulation efficiency of PZQ was about 80%. *In vitro* drug release fitted the Weibull distribution equation. There were two peaks in the PZQ concentration-time curves in plasma after oral administration of PZQ-SLN. The first peak might be caused by free drug and that adsorbed onto the surface of PZQ-SLN. The second peak was indicative of gut uptake of PZQ-SLN. The $AUC_{0\rightarrow\infty}$ value of PZQ after oral administration of SLN was 4.1 fold higher than that obtained with the PZQ-TAB. The MRT of PZQ-SLN was also significantly enhanced, resulting in an about twofold increase compared with PZQ-TAB. Thus, the oral bioavailability of PZQ-SLN increased significantly compared to PZQ-TAB, and the results indicate that SLN can be a promising drug carrier for PZQ.

1. Introduction

Praziquantel (PZQ) is the drug of choice in the treatment of schistosomiasis. The failure of mass treatment to control schistosomiasis has been attributed to the fact that PZQ is extensively converted into inactive or considerably less potent compounds after oral administration (Dayan 2003).

Many efforts have been made to increase the bioavailability of PZQ. The concomitant administration of cimetidine or food increases the plasma levels of PZQ, with an improvement in treatment outcome (Jung et al. 1997; Castro et al. 2000, 2002). Other studies described the improvement of dissolution rate by adjuvants such as β-cyclodextrin and polyvinylpyrrolidone (El-Arini and Leuenberg 1998; Becket et al. 1999). Another attempt of improving the effectiveness of PZQ is the use of liposomes (Mourao et al. 2005) and PLGA nanoparticles (Mainardes and Evangelista 2005) in intravenous injections.

The oral bioavailability of poorly water soluble drugs can be improved when these drugs are encapsulated in lipid-based vehicles (Humberstone and Charman 1997). The main mechanisms of uptake of particulate materials across the intestine are Peyer's patches, intracellular uptake, paracellular passage or mixed uptake via both routes (Lavelle et al. 1995). However, the translocation via the uptake in Peyer's patches seems to be a major pathway after oral administration of nanoparticles. Thus, the nanoparticles loaded with drugs could avoid first pass hepatic metabolism and reach a reduction in the dose delivered. Solid lipid nanoparticles (SLN) were introduced at the beginning of the 1990s, since they have all the advantages of fat emulsions (large scale production, no organic solvents, low systemic and cytotoxicity) and polymeric nanoparticles (controlled drug release due to a solid lipid matrix) (Schwarz and Mehnert 1999; Mehnert and Mader 2001). They open a broad field of applications including i.v., oral and dermal administration. Taking into account the potential of SLN nanoparticles to improve the bioavailability of lipophilic drugs (Hu et al. 2004), the aim of this work was to prepare SLN containing PZQ by ultrasonic dispersion technique and assess the feasibility of SLN to enhance the oral bioavailability of PZQ.

2. Investigations, results and discussion

2.1. Characterization of PZQ-SLN

In this study, reproducible PZQ-SLN with a trapping efficiency of 79.3 ± 0.69% was prepared. TEM micrographs (Fig. 1) showed that the particles had nearly round shapes. The PZQ-SLN had a mean size of 110 nm with a narrow size distribution of 19 nm (Fig. 2) and the zeta potential of the sample was −66.3 mv which may contribute to their physical stability. These results showed that fine SLN loaded with PZQ can be readily and quickly prepared by the ultrasound technique.

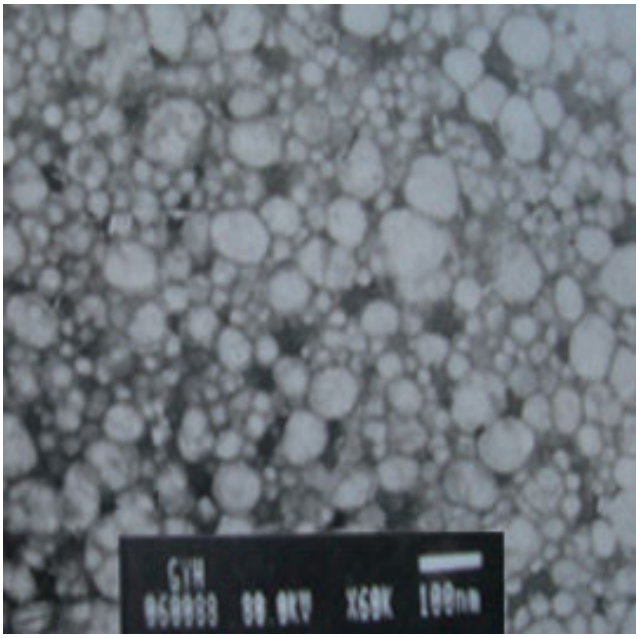

Fig. 1: PZQ-SLN under transmission electron microscopy (× 60 K)

2.2. *In vitro release*

Drug release was studied using diffusion cells separating the receptor fluid and the nanoparticle dispersion. *In vitro* release curves for drug-loaded nanoparticles are shown in Fig. 3. The drug release data from PQ-SLN in different pH dissolution media fitted Weibull distribution equation. The correlation coefficient of the equation was 0.9973. In different pH dissolution media, a biphasic drug release pattern was observed, drug burst release at the initial stage followed by sustained release at a constant rate. At the initial stage, the drug released most fast from SLN. One possible explanation was due to the adsorbed PZQ on the surface of nanoparticles. During the cooling process from the melted lipid droplet in dispersed medium to the formation of a SLN at room temperature, because of the different solubility of PZQ at different temperatures, the surplus

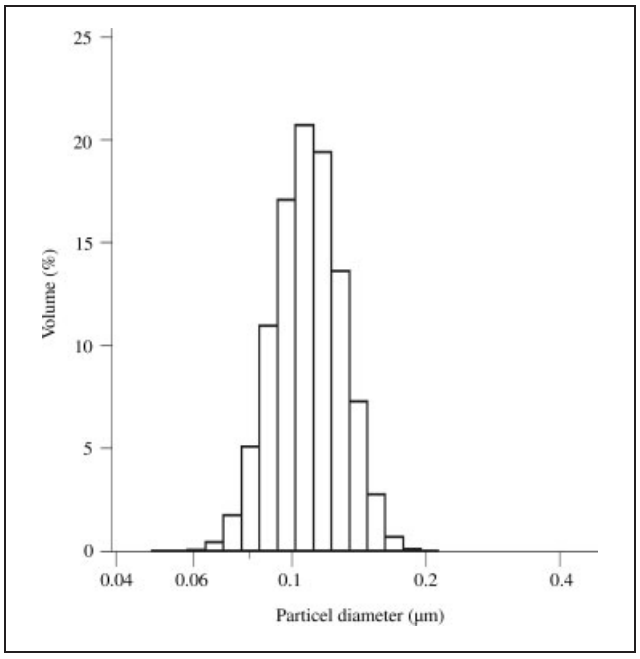

Fig. 2: Size distribution of PZQ-SLN

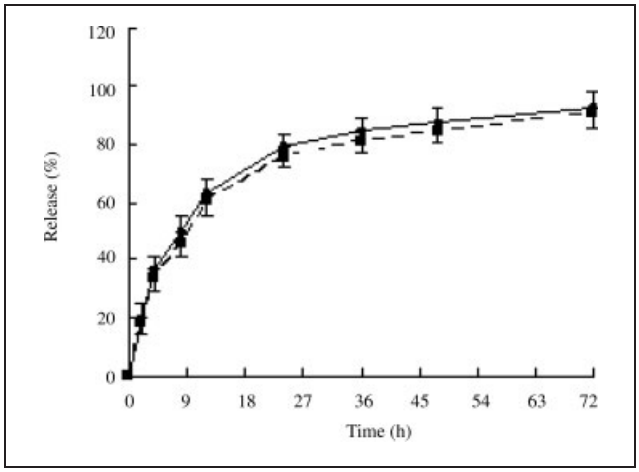

Fig. 3: *In vitro* release profiles of PZQ-SLN in 3.0% SDS artificial gastric juice (□———□) and 3.0% SDS artificial enteric juice (■- - - - -■) (n = 3)

PZQ located at the outer shell of the nanoparticles which led to drug-enriched shell related with drug burst release at the initial stage. Therefore, the SLN showed burst release at the initial stage and subsequently sustained release, although the pH of dissolution medium was different.

2.3. *In vivo pharmacokinetic study*

A sensitive reversed-phase HPLC was used for the detection of PZQ in plasma after oral administration. The lowest detectable limit of PZQ was 8.4 ng · mL^{-1}. There were no endogenous components that interfered with detection of PZQ. The relative recoveries at low, medium and high concentrations were between 95.0% and 98.0% (n = 3). The standard curves having PZQ concentrations ranging from 0.0246 to 8.0 μg · mL^{-1} exhibited good linearity, and correlation coefficients over this concentration range were 0.9971 (n = 3). The assay was accurate and reproducible with coefficients of variation ranging from 3.6% to 5.7%.
The oral concentration-time curves after a single dose of PZQ-TAB and PZQ-SLN in rats are shown in Fig. 4. The oral pharmacokinetic parameters are listed in the Table. There were two peaks in the concentration-time curve of PZQ-SLN, respectively. The C_{max} values of PZQ in SLN were 2.4 μg · mL^{-1} and 2.59 μg · mL^{-1}, respectively, compared with 2.14 μg · mL^{-1} of that in tablets. The first peak was caused by free and adsorbed drug because PZQ will be very quickly absorbed in the gastrointestinal tract (Dayan 2003). The second increase in the plasma concen-

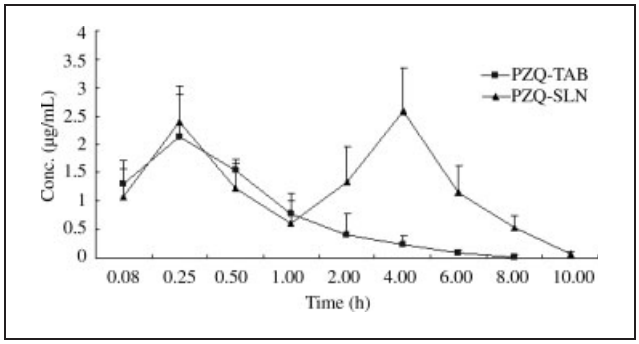

Fig. 4: Mean concentration-time curve after a single oral administration of PZQ-TAB and PZQ-SLN in rats (60 mg · kg^{-1}). Data are means ± S.D., n = 5.

Table: Pharmacokinetic parameters of PZQ after oral administration of PZQ-TAB and PZQ-SLN (n = 5)

Samples	C_{max} ($\mu g \cdot mL^{-1}$)	T_{max} (h)	K_e (h^{-1})	$T_{1/2}$ (h)	$AUC_{0-\infty}$ ($\mu g \cdot h \cdot mL^{-1}$)	$AUMC_{0-\infty}$ ($\mu g \cdot h^2 \cdot mL^{-1}$)	MRT (h)	Cl ($L \cdot h^{-1}$)	Vss (L)
PZQ-TAB	2.14	0.25	0.412	1.859	3.084	6.370	1.999	3.573	6.906
PZQ-SLN	2.4, 2.59	0.25, 4	0.557	1.253	12.361	50.605	4.111	1.048	4.324

Data are means, $^*P < 0.05$ compared with PZQ-TAB

tration-time profile after 1 h might be due to the translocation of SLN across the gastrointestinal tract. The $AUC_{0\rightarrow\infty}$ value of PZQ after oral administration of SLN was 4.1 fold higher than that obtained with the PZQ-TAB. The MRT of PZQ-SLN was also significantly enhanced, resulting in an about twofold increase compared with PZQ-TAB. From these results, it can be concluded that the oral bioavailability of PZQ is significantly enhanced by SLN compared with tablets, and SLN can be a promising sustained release system.

Although many techniques have been employed to improve the oral bioavailability of PZQ, most of them are used to enhance the dissolution of PZQ. But it is not enough to improve its dissolution, as long as the released PZQ cannot bypass the liver first passage. Many publications have reported that nanoparticles can avoid the first pass hepatic metabolism. So we carried out detailed studies of SLN for improving the oral bioavailability of PZQ.

The uptake and transport of SLN (average diameter 80 nm) in the lymph and blood were evidenced after duodenal administration in rats (Bargoni 1998). The quantity of gut uptake of nanoparticles and their translocation to organs seems to strongly depend on their size, nature of the particulate, hydrophobicity and surface charge (Florence et al. 1995). Desai et al. (1996) have disclosed that there was a microparticle size dependent exclusion phenomena in the gastrointestinal mucosal tissue with 100 nm size particles showing significantly greater tissue uptake. Due to their small particle size, SLN may exhibit bioadhesion to the gastrointestinal tract wall or enter the intervillar spaces thus increasing their residence time in the gastrointestinal tract. This increase in adhesion will result in enhanced bioavailability. Another advantage of SLN formulations over PZQ-TAB is the lipid protection from enzymatic degradation, thereby delaying *in vivo* metabolism. By incorporation into nanoparticles, PZQ can be embedded into a solid lipid matrix thus reducing its exposure to enzymatic degradation.

Absorption of intact PZQ-SLN might exist according to the change of pharmacokinetic parameters of PZQ-SLN and PZQ-TAB. The higher bioavailability and longer MRT may be due to an enhanced lymphatic uptake in comparison with PZQ-TAB, similar increase in bioavailabily due to entrapment of the drug into SLN which was previously reported for camptothecin (Yang 1999). As compared with the PZQ-TAB, the higher MRT value, AUC indicated that PZQ-SLN could much improve drug treatment efficency.

2.4. Conclusions

In our study, a poorly aqueous-soluble drug PZQ was successfully incorporated into SLN by the ultrasound technique. The physicochemical characteristics were investigated. The SLN showed burst release at the initial stage and sustained release subsequently. An oral pharmacokinetic study was conducted in male rats and the results showed that SLN produced a significant improvement in the bioavailability of PZQ compared with PZQ-TAB. It appears that SLN offer a promising drug delivery system for the enhancement of the bioavailability of poorly soluble drugs like PZQ.

3. Experimental

3.1. Materials

Compritol 888 ATO (Co, Chengdu Tianlu Chemical Reagent Factory, China) and butyl acetate (Shenyang Chemical Reagent Factory, China) were used as lipid materials of SLN. PZQ was obtained from Shanghai Bangcheng Chemical Co. Ltd, China. PZQ-TAB were kindly presented by Schistosomiasis Prophylactio-therapeutic Institution of Wuxue, Hubei Province, China. Poloxamer 188 (Shenyang pharmaceutical university factory, China), sodium stearate (Wenzhou Dongsheng Chemical Reagent Factory, China) and soybean lecithin (PC, Shanghai Jinban pharmaceutical Co., Ltd, China) were used as dispersing agents in water phase. Ethanol and acetone were of chromatographic grade.

3.2. Preparation of SLN

The basic formulation contained 4.6% lipid matrix, 4.0% poloxamer, 0.2% sodium stearate, 4.0% PC, 0.4% PZQ and 86.8% bidistilled water, which were all w/v unless mentioned otherwise. Both butyl acetate and PZQ were weighted into containers. The mixtures were stirred with a Teflon coated magnet at 70 °C until a transparent yellow solution was obtained. The left components were weighed into containers and heated up to 70 °C. Then the aqueous phase was directly poured into the oil phase. The hot mixer was immediately subjected to autosonic treatment for 15 min using a high-intensity probe ultrasonicator (600 W; Ningbo Schizh Inc., China) at 70 °C. Subsequently, the dispersions were allowed to recrystallize at room temperature. The dispersions were filtered through a 0.22 μm membrane and the final volume was adjusted to 100 mL with distilled water.

3.3. Characterization of PZQ-SLN

3.3.1. Particle size analysis

Particle size analysis was performed by photon correlation spectroscopy (PCS) using the laser light scattering instrument (LS230; BECKMAN COULTER) at 25 °C. The particle size analysis data were evaluated using the number distribution. The dispersions were diluted with distilled water for size determination.

3.3.2. Zeta potential analysis

Zeta potential measurements of PZQ-SLN were carried out using Zeta potential analyzer (Delsa 440SX; BECKMAN COULTER); SLN dispersions in water were diluted 1 : 40 with distilled water (v/v) before analysis.

3.3.3. Transmission electron microscopy (TEM)

The morphology of SLNs was examined using an transmission electron microscope (TEM-1200EX; Japan). The SLNs were dispersed directly into the distilled water. Then Cu grid coated with C film was put into the above solution several times. After being stained by 2% phosphotungstic acid (PTA) solution and dried under room temperature, the sample was ready for the TEM investigation.

3.4. Drug entrapment efficiency (EE%)

After adding 0.2 mL nanosuspension solution to a Sephadex G-25 microcolumn prepared by us, 0.2 mL distilled water passed through the microcolumn which was centrifuged for 2.0 min at 500 rpm. All the manipulation was performed 3 times. The collected washing out liquid with opalescence was the nanosuspension without free drug, which was dissolved in heated ethanol solution, then PZQ was separated from the lipid matrix at room temperature. At last the amount of drug that was incorporated into the SLNs ($W_{contained}$) could be obtained by HPLC using an Jasco PU-1580 pump, an Jasco UV-1575 variable wavelength detector set at 263 nm. An Diamonsil® C18 column (150 mm × 4.5 mm) was used. The mobile phase consisted of methanol and water (70 : 30, v/v). Another 0.2 mL SLN dispersion was directly metered volume to 5 mL, and HPLC was used as described above to determine the total amount of PZQ (W_{total}).

Entrapment efficiency (EE%) could be achieved by the following equation.

$$EE(\%) = W_{contained}/W_{total} \times 100\%.$$

$W_{contained}$: amount of PZQ incorporated in the SLNs, W_{total}: total amount of PZQ including free and incorporated PZQ in formulation.

3.5. In vitro release study

The *in vitro* drug release profiles from SLN were investigated using horizontal diffusion cells (1.4 cm in diameter). The diffusion cells were thermoregulated with a thermostat-controlled water-bath at 37 °C. Cellulose membrane with a molecular weight cut off of 1,2000 ~ 1,4000 separated donor and receptor fluid. Artificial gastric juice containing 3.0% (w/v) sodium dodecyl sulphate (SDS) was used as receptor fluid, as well as artificial enteric juice containing 3.0% SDS. Liquid nanoparticle dispersion (1 mL) containing PZQ and 9 mL dissolution medium were applied on the donor side. Pure dissolution medium (10 mL) was used on the receptor side. 1 mL dispersion was withdrawn from the receptor side at each time interval over 72 h, then added with the same volume of fresh dissolution medium each time. The samples were determined by HPLC as described above. Data represent the arithmetic mean ± standard deviation (S.D.) (n = 3).

3.6. Bioavailability and pharmacokinetic study after oral administration

3.6.1. Oral administration

Male Wistar rats (provided by Shenyang Pharmaceutical University Animals Center, 200 ± 20 g) were used for the oral administration study. During the tests, all the rats were fasted overnight. After the single oral dose (60 mg · kg^{-1}) of PZQ-TAB as control or PZQ-SLN, the fast continued for further 4 h and the rats had free access to water. At predetermined time intervals, blood samples were drawn from the caudal vein into heparinized tubes and separated immediately by centrifugation, then the plasma was stored at −20 °C until analysis.

3.6.2. HPLC analysis of PZQ

Samples were analyzed by an HPLC method previously reported with little modification (Warunee Hanpitakpong et al. 2004). Briefly, to 0.2 mL plasma, 200 ng internal standard working solution (diazepam 20 μg · mL^{-1}) were added. The samples were mixed in a vortex for 4–5 s and extracted with 1.2 mL methyl-*tert*-butylether/dichloromethane mixture (2 : 1, v/v). After being subjected to vortex for 3.0 min, the organic layer was separated by centrifugation at 1500 × *g* for 10 min. The upper organic layer (0.9 mL) was transferred to a clean tube and evaporated to dryness under a stream of nitrogen at 40 °C. The residue was dissolved in 100 μL of mobile phase and 20 μL were injected onto the HPLC column.
The HPLC system consisted of a Jasco HPLC solvent Delivery/Controller, equipped with a PU 1580 pump and a 1575 ultraviolet detector. The wavelength was set at 217 nm. The separation was carried out on a reversed phase column Diamonsil® C18 (150 mm × 4.6 mm, 5 μm particle size: Dikma, China). The mobile phase consisted of methanol, acetonitrile and distilled water (25 : 40 : 60, v/v), running through the column at a flow rate of 1.0 mL · min^{-1}. The chromatographic analysis was operated at 40 °C.

3.6.3. Calibration curves

Calibration curves were prepared by linear regression analysis of ten blank plasma samples (0.2 mL each) added with varying concentrations of PZQ covering the range of 0.0246–8.0 μg · mL^{-1} and a fixed concentration of the internal standard (20 μg· mL^{-1}). Samples were analyzed as described above.

3.6.4. Data analysis

The pharmacokinetic parameters were calculated by a non-compartmental method. The area under the concentration-time curve from time zero to time t ($AUC_{0\to t}$) was calculated using the trapezoidal method. Peak concentration (C_{max}) and time of peak concentration (T_{max}) were obtained directly from the individual plasma concentration-time profiles. The area under the total plasma concentration-time curve from time zero to infinity was calculated by

$$AUC_{0\to\infty} = AUC_{0\to t} + C_t/K_e$$

where C_t is the PZQ concentration observed at last time, and K_e is the apparent elimination rate constant obtained from the terminal slope of the individual plasma concentration-time curves after logarithmic transformation of the plasma concentration values and application of linear regression. The relative bioavailability F_r at infinity at the same dose was calculated as

$$Fr = AUC_{PZQ-SLN,0\to\infty}/AUC_{PZQ-TAB,0\to\infty}.$$

The area under the first moment curve ($AUMC_{0\to\infty}$) was also calculated using the linear trapezoidal rule. The mean residence time (MRT) was determined by dividing $AUMC_{0\to\infty}$ by $AUC_{0\to\infty}$.

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