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Journal of Controlled Release 114 (2006) 53–59
journal of controlled release
www.elsevier.com/locate/jconrel

Solid lipid nanoparticles for enhancing vinpocetine's oral bioavailability

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Received 26 December 2005; accepted 17 May 2006
Available online 23 May 2006

Abstract

An ultrasonic-solvent emulsification technique was adopted to prepare vinpocetine loaded Glyceryl monostearate (GMS) nanodispersions with narrow size distribution. To increase the lipid load the process was conducted at 50 °C, and in order to prepare nanoparticle using an ultrasonic-solvent emulsification technique. The mean particle size and droplet size distribution, drug loading capacity, drug entrapment efficiency (EE%), zeta potential, and long-term physical stability of the SLNs were investigated in detail respectively. Drug release from two sorts of VIN-SLN was studied using a dialysis bag method. A pharmacokinetic study was conducted in male rats after oral administration of 10 mg kg^{-1} VIN in different formulations, it was found that the relative bioavailability of VIN in SLNs was significantly increased compared with that of the VIN solution. The amount of surfactant also had a marked effect on the oral absorption of VIN with SLN formulations. The absorption mechanism of the SLN formulations was also discussed. These results indicated that VIN absorption is enhanced significantly by employing SLN formulations. SLNs offer a new approach to improve the oral bioavailability of poorly soluble drugs.
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Keywords: Solid lipid nanoparticles; Glyceryl monostearate; Ultrasonic-solvent emulsification technique; In-vitro; In-vivo

1. Introduction

SLNs have been reported as an alternative drug delivery system to traditional polymeric nanoparticles [1]. A clear advantage of solid lipid nanoparticles (SLNs) over polymeric nanoparticles is the fact that the lipid matrix is made from physiologically tolerated lipid components, which decreases the potential for acute and chronic toxicity [2]. At room temperature the particles are in the solid state [3,4]. SLNs combine the advantages of polymeric nanoparticles, fat emulsions and liposomes [5]. They can be produced on a large industrial scale by high-pressure homogenization [6], with low toxicity potential like emulsions and liposomes, produce sustained release due to their solid matrix, similar to polymeric nanoparticles, and can effectively target specific tissues after parenteral administration [7,8].

The process of solvent-evaporation method is based on the water immiscible solvents [9,10]. Upon transferring a transient oil-in-water emulsion into water, the drug dissolved in the organic solvent solidifies instantly due to diffusion of the organic solvent from the droplets to the continuous phase. But the SLNs prepared by this method always have large particle diameter and wide size distribution. The aim of this study was to investigate the feasibility of preparing glyceryl monostearate nanoparticles with different emulsifiers using ultrasonic-solvent emulsification technique to prepare drug loaded SLNs with particle diameter below 100 nm and low polydispersity.

Vinpocetine (VIN), the chemical structure of which is shown in Fig. 1, has been shown to improve cerebral circulation and metabolism in the treatment of various types of cerebrovascular circulatory disorder, e.g. cerebral infarction (CI), cerebral hemorrhage residual and cerebral arteries cirrhosis, etc. Due to its poor aqueous solubility and extensively metabolized during first pass, its clinical use is greatly restricted by the low bioavailability after oral administration [11] and so there is a need to improve its poor aqueous solubility to increase its oral bioavailability. An oral formulation with a high degree of oral absorption would, therefore, be highly desirable.

In this study, VIN-loaded SLNs were successfully prepared by an ultrasonic-solvent emulsification technique and the physicochemical characteristics of the SLNs were investigated.

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doi:10.1016/j.jconrel.2006.05.010

Drug release from the SLNs was studied and compared with the diffusion from a VIN solution using a dialysis bag method. The oral bioavailability of VIN in SLNs investigates the absorption enhancement of SLNs for poorly soluble drugs. In addition, the effect of surfactant on the oral absorption of VIN was also studied. The behaviour and absorption mechanism of the SLN formulations were discussed.

2. Materials and methods

2.1. Materials

VIN was supplied by DongBei Pharmaceutical Co. (Shenyang, China). Glyceryl monostearate (GMS) was purchased from ChangSha Chemical Co. (Hunan, China). Soya lecithin was provided by Shanghai TaiWei Pharmaceutical Co. (Shanghai, China). Polyoxyethylene hydrogenated castor oil was kindly donated by BASF (Luwigshafen, Germany). Tween 80 and dichloromethane were obtained from Shenyang Chemical Reagent Factory (Shenyang, China). All other chemicals were analytical reagent grade and used without further purification.

2.2. Preparation of VIN-SLNs

SLNs were prepared by an ultrasonic-solvent emulsification technique. The desired amounts of GMS and VIN were mixed with a sprinkle of dichloromethane and heated to 50 °C, emulsifiers (soya lecithin/Tween 80 or soya lecithin/Polyoxyethylene hydrogenated castor oil) were dispersed in 10 mL distilled water with magnetic stirring at the same temperature. After evaporating most of the dichloromethane, the water phase was added to the oil phase by drop-by-drop at 50 °C followed by magnetic stirring for 10 min, then the coarse emulsion was subjected to 600 W of ultrasonic treatment for 5 min using a high-intensity probe ultrasonicator (JY92-2D; Xinzhi Equ.Inst., China) with water bath (0 °C). The dispersions were immediately dispersed in bulks of distilled water (0 °C) followed by magnetic stirring to remove traces of organic solvents if any. After the dichloromethane had completely evaporated (data was not shown), the VIN-SLN suspensions were filtered through a 0.45 μm membrane in order to remove the impurity materials (e.g. metal) carried in when ultrasonication and then storage at 4 °C.

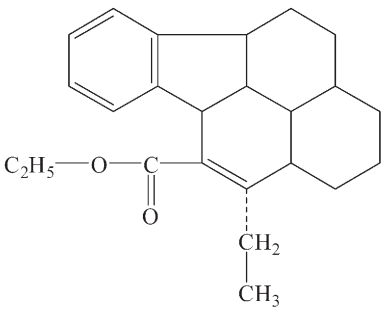

Fig. 1. The structure of vinpocetine.

Table 1
Composition of formulations for the oral administration study (wt.%)

Ingredient	SLN A	SLN B	SLN C	SLN D	SLN E	SLN F
VIN	0.1	0.1	0.1	0.1	0.1	0.1
GMS	3.0	3.0	3.0	3.0	3.0	3.0
Tween 80	2.0	1.5	1.0			
Polyoxyethylene hydrogenated castor oil				2.0	1.5	1.0
Soya lecithin	2.0	2.0	2.0	2.0	2.0	2.0
Dichloromethane	Trace	Trace	Trace	Trace	Trace	Trace
Water	92.9	93.4	93.9	92.9	93.4	93.9

All the formulations used were shown in Table 1.

2.3. Drug content and entrapment efficiency

One milliliter SLN dispersion was separated by Sephadex G-50 column (SINO-AMERICAN BIOTECHNOLOGY Co.; America). The parts of the outflow with opalescence and metered volume to 25 mL were collected, then 2 mL of which was dissolved in 3 mL tetrahydrofuran and added eluant to 10 mL, the lipid was preferentially precipitated by vortexing. After centrifugation (4000 rpm^{-1} for 15 min), the drug content in the supernatant was measured by HPLC. The HPLC system consisted of a mobile phase delivery pump (LC-10AD; SHIMADZU, Japan), a UV–VIS detector (SPD-10A; SHIMADZU, Japan) and a 20 μL loop (Rhenodyne model 7725i). A C_{18} reverse-phase column (Hypersil ODS C_{18}, 200×4.6 mm; Dalian, China) and a Phenomenex C_{18} security guard (4 mm×3.0 mm, 5 μm, Torrance) were utilized for drug separation, using methanol–0.01 M aqueous $(NH_4)_2CO_3$ (83:17, v/v) as mobile phase. The flow rate and UV wavelength were 1.0 mL min^{-1} and 273 nm, respectively.

Another 1 mL SLN dispersion was metered volume to 25 mL directly and then 2 mL of which was treated and analyzed as described above.

The equations for the drug content and loading efficiency are as follows:

$$\text{Entrapment efficiency } (\%) = \frac{W_S}{W_{total}} \times 100\%$$

$$\text{Load content } (\%) = \frac{W_S}{W_{lipid}} \times 100\%$$

W_s: amount of VIN in the SLNs; W_{total}: amount of VIN used in formulation: W_{lipid}: weight of the vehicle.

2.4. Characterization of VIN-SLNs

2.4.1. Particle size and zeta potential

The mean diameter of SLNs in the dispersion was determined by photon correlation spectroscopy (PCS) using a laser light scattering instrument (LS230; COULTER) at a fixed angle of 90° at 25 °C [12]. The particle size analysis data was evaluated using the volume distribution. Zeta potential

measurement was carried out using Zeta potential analyzer (Delsa 440SX; BECKMAN COULTER) at the same temperature. Before measurement, SLN dispersions were diluted 50-fold with the original dispersion preparation medium for size determination and zeta potential measurement. All the measurements were performed in triplicate.

2.4.2. Transmission electron microscopy (TEM)

The morphology of SLNs was examined using an electronic transmission microscope (Hitachi H-100; Japan). After diluting 50-fold with the original dispersion medium of the preparation, the samples were negatively stained with 1.5% (w/v) phosphotungstic acid for observation.

2.4.3. Stability studies

Two formulations (SLN A and SLN D) were injected into 10 mL ampoules and sealed for storage at 4 °C for 1 year. The average size, zeta potential and entrapment efficiency were determined.

2.5. Drug release properties of VIN-SLNs

The drug release from VIN-SLN A, VIN-SLN B and VIN-Sol (purchased from Zhengzhou LingRui Drug Co.) was performed in 0.1 M HCl, distilled water (pH 6.5) and phosphate buffer (pH 7.4), respectively, using the dialysis bag method. The dialysis bag retains nanoparticles and allows the free drug into the dissolution media with a cut-off of 8000–14 000. The bags were soaked in double-distilled water for 12 h before use. Two milliliters of SLN dispersion (1.0 mg mL^{-1}) was poured into the bag with the two ends fixed by clamps. The bags were placed in a conical flask and 50 mL receiving phase was added. The conical flasks were placed into a thermostatic shaker (HZQ-C; Haerbin Dongming Medical Instrument Factory, Haerbin, China) at 37 °C at a rate of 140 times per min. At 1, 2, 4, 8, 12, 24, 36, 48, 60, 72, 96 h after test, the medium in the conical flask was completely removed by filtration for analysis and fresh dialysis medium was then added to maintain sink conditions. The filtrate was analyzed by the HPLC method. All the operations were carried out in triplicate.

2.6. Oral administration

Male Wistar rats (provided by China Medical University Animals Center), 250±20 g were used for the oral administration study . All animal experiments complied with the requirements of the National Act on the use of experimental animals (People's Republic of China). All the rats were fasted for 12 h before the experiments but had free access to water. After light anaesthetization with ether, the suspension of VIN-SLN A, VIN-SLN B and VIN-Sol (purchased from Zhengzhou LingRui Drug Co.) administered to 6 rats by gavage at a VIN dose of 10 mg kg^{-1}. Blood (0.5 mL) was collected via the caudal vein at 0, 0.25, 0.50, 1, 1.5, 2, 3, 4, 6, 8 and 12 h after administration separately. Blood samples were placed into heparinized tubes and separated immediately by centrifugation. After centrifugation, the plasma obtained was stored at −20 °C until analysis.

2.7. Quantification of plasma concentration

VIN plasma concentrations were determined by HPLC analysis as described above. A 200 μL plasma sample was placed into a centrifuge tube and 5 μL internal standard (apovincaminic acid methylester, AVAM) and 100 μL 0.5 M NaOH were added then extracted in stoppered test tubes with 600 μL of diethyl ether under vigorous shaking at room temperature for 30 s. After centrifugation at 4000 rpm^{-1} for 15 min, the ether phase was aspirated and the extraction procedure was repeated with another 600 μL of diethyl ether. Ether phases were collected and evaporated to dryness under air stream. Then 100 μL methanol was used to dissolve the residue and injected for HPLC analysis. Calibration curves were prepared by linear regression analysis of the plot of the peak area against the concentration of VIN covering the range of 50–8000 ng mL^{-1}. The concentration of plasma samples was determined from the area of the chromatographic peak using the calibration graph. The limit of detection was 50 ng mL^{-1}.

2.8. Data analysis

The pharmacokinetic parameters were calculated based on a non-compartmental model. The area under the concentration–time curve from time zero to time t ($AUC_{0\rightarrow t}$) 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: $AUC_{0\rightarrow\infty}=AUC_{0\rightarrow t}+C_t/K_e$, where C_t is the VIN 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: $F_r=AUC_{sln,\ 0\rightarrow\infty}/AUC_{sol,\ 0\rightarrow\infty}$. The mean residence time (MRT) was estimated from $MRT=AUMC_{0\rightarrow\infty}/AUC_{0\rightarrow\infty}$.

The data obtained from the release rate and pharmacokinetic parameters were analyzed statistically by one-way analysis of variance and t-test using a statistical package for social sciences (SPSS version 11.0) software. Statistically significant differences were assumed when $P<0.05$. All values are expressed as their mean±S.D.

3. Results and discussion

3.1. Characterization of SLNs

The mean particle size of VIN-SLN prepared with different formulations, ranged from 70 to 200 nm (Table 2). The SLN formulations containing Polyoxyethylene hydrogenated castor oil or Tween 80 showed a relatively small size distribution.

Table 2
The mean diameter, zeta potential and entrapment efficiency of different SLN formulations (0 day)

Formulation	Mean diameter (nm)	Zeta potential (mV)	Entrapment efficiency (%)
SLN A	70.3±7.8	−33.8±0.9	97.2±0.3
SLN B	100.2±9.3	−29.7±0.6	96.9±0.4
SLN C	148.6±11.8	−26.3±1.2	98.1±0.6
SLN D	77.8±9.0	−29.6±1.1	98.3±0.4
SLN E	113.4±8.5	−26.7±0.7	98.2±0.5
SLN F	167.6±5.8	−22.4±0.5	98.7±0.3

The results show that VIN has a high entrapment efficiency in SLNs. When the amounts of the emulsifiers increased, the entrapment efficiency decreased due to the solubilization of the emulsifiers. The entrapment efficiency of formulations containing Tween 80 was a little lower than that of formulations containing Polyoxyethylene hydrogenated castor oil. These results may have contributed to the higher solubilization of Tween 80 compared with Polyoxyethylene hydrogenated castor oil in the aqueous system for VIN.

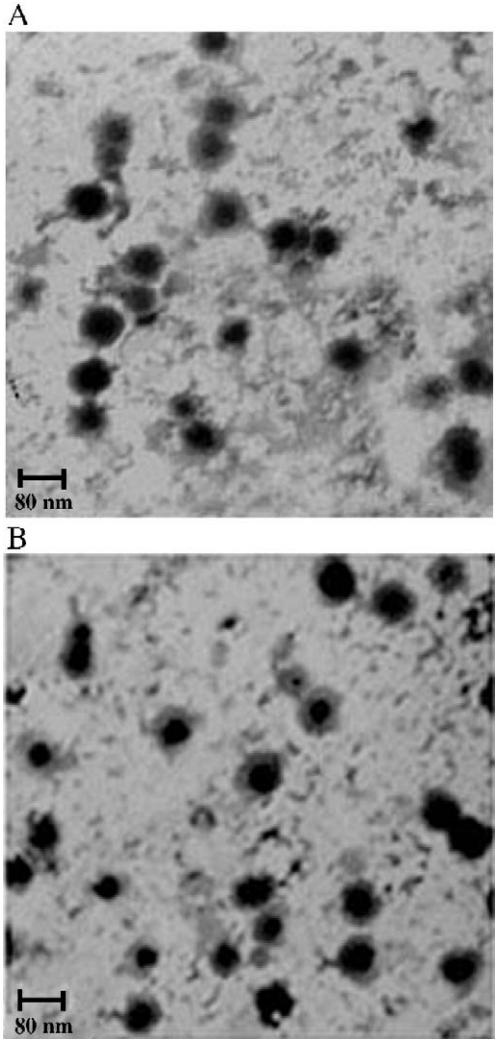

Fig. 2. Micrographs of SLN by transmission electron microscope. (A: SLN A; B: SLN D).

The SLNs had a zeta potential ranging from −17 mV to −40 mV. As the amount of Polyoxyethylene hydrogenated castor oil and Tween 80 increased, the zeta potential decreased significantly.

TEM shows that the particles had round and uniform shapes. The mean diameters of SLN A and SLN D were in the range of approximately 60–90 nm and 70–100 nm, respectively (Fig. 2).

3.2. Stability data

After 1 year of storage at 4 °C, no dramatic increase in the size of the SLN A and SLN D occurred (Table 3). The entrapment efficiencies of all the SLN formulations had fallen by about 2%. This result may be due to the crystal transition of saturate fatty glyceride which was caused by the temperature and storing [13]. Other SLN formulations showed similar results (data not shown).

3.3. Drug release of VIN-SLNs

VIN release from the two SLN formulations and diffusion from VIN solution are shown in Fig. 3A–C. The rate at which VIN was released from SLN A and SLN D was affected by the pH of the dissolution medium, the release rate increased as the pH decreased. From the experimental data, we can see that in the same dissolution media, SLN A always released VIN faster than SLN D. The difference was significant for 96 h with SLN A and SLN D. This may be due to the stronger solubilization of Tween 80 compared with Polyoxyethylene hydrogenated castor oil. The rate of VIN solution was slow in all three media. From these results, we concluded that the surfactants (Tween 80 and Polyoxyethylene hydrogenated castor oil) made an important contribution to the differences between the release from the two SLN formulations and diffusion from VIN solution. Surfactants diffused into the receiver side, altered the barrier properties of the aqueous boundary layer and the permeability of the membrane, resulting in a high release velocity of VIN in SLN dispersion. The VIN solution would not have this effect. In addition, the concentration of VIN in SLN dispersion was close to saturation (maximal thermodynamic activity), while in VIN solution, although the overall concentration of VIN was identical with that in SLN dispersion, with the appearance of microcrystals the real concentration of drug dissolved in solution would be greatly lowered, since thermodynamic

Table 3
Effects of storage time (at 4 °C) on particle size and entrapment efficiency of two SLN formulations ($n=3$, $\bar{x}\pm s$)

SLN	Size (nm)	Zeta potential (mV)	Entrapment efficiency (%)
SLN A			
0 day	70.3±7.8	−33.8±0.9	97.2±0.3
6 months	82.8±5.6	−32.3±1.1	96.1±0.6
12 months	120.2±10.5	−29.1±0.5	95.3±0.4
SLN B			
0 day	77.8±9.0	−29.6±1.1	98.3±0.4
6 months	89.8±6.2	−28.3±0.9	97.4±0.5
12 months	126.6±5.4	−26.8±1.2	96.3±0.4

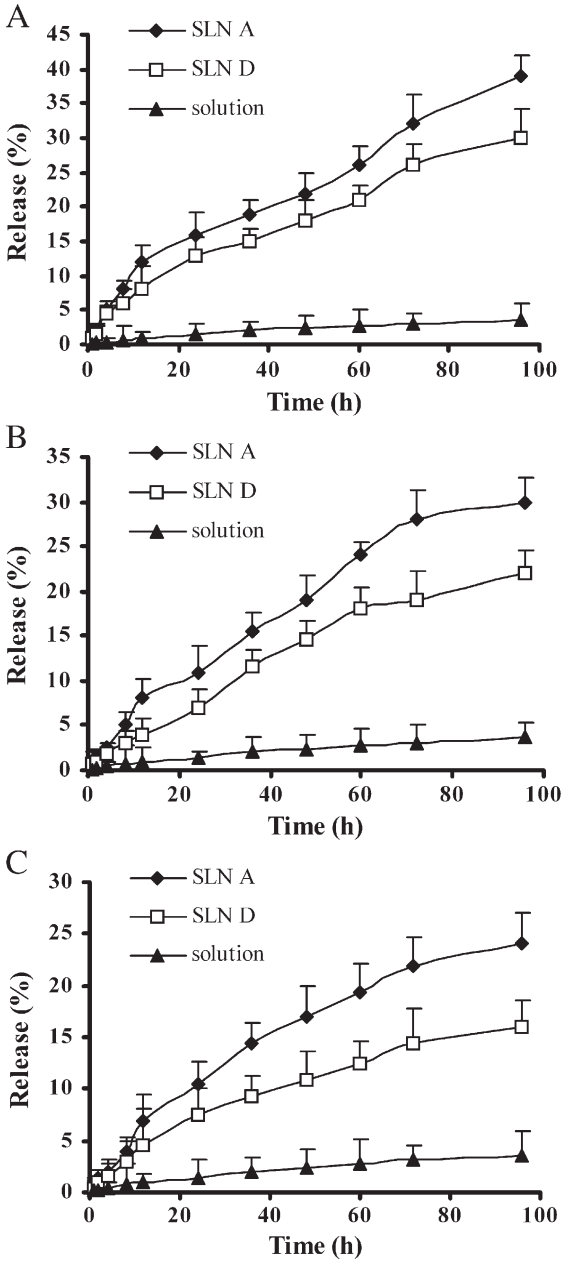

Fig. 3. In-vitro release of VIN from SLN A, SLN D and VIN solution in three different dissolution media: 0.1 M HCl (A); distilled water, pH 6.5 (B) and phosphate buffer, pH 7.4 (C). Data are means±S.D., $n=6$.

activity is the driving force for transport, so the diffusion of VIN into the receiver side was slow compared with that in the SLN dispersion.

3.4. In-vivo pharmacokinetic study

The oral concentration–time curve after a single dose of different VIN formulations in rats is shown in Fig. 4A–C. The oral pharmacokinetic parameters are listed in Table 4.

Fig. 4A shows the concentration–time curve of SLN A, SLN D, VIN solution. At all time points, the VIN plasma concentrations were significantly higher for rats treated with VIN-SLN than for those treated with VIN solution. The C_{max} values of VIN in SLN A and SLN D were higher (3.00 μg mL^{-1} and 2.40 μg mL^{-1}, respectively) than that obtained with the solution (0.75 μg mL^{-1}). Twelve hours after oral administration, the VIN plasma concentrations were still 0.10 μg mL^{-1} and 0.15 μg mL^{-1}, respectively, whereas they were undetectable 6 h after administration of VIN solution. Incorporation into

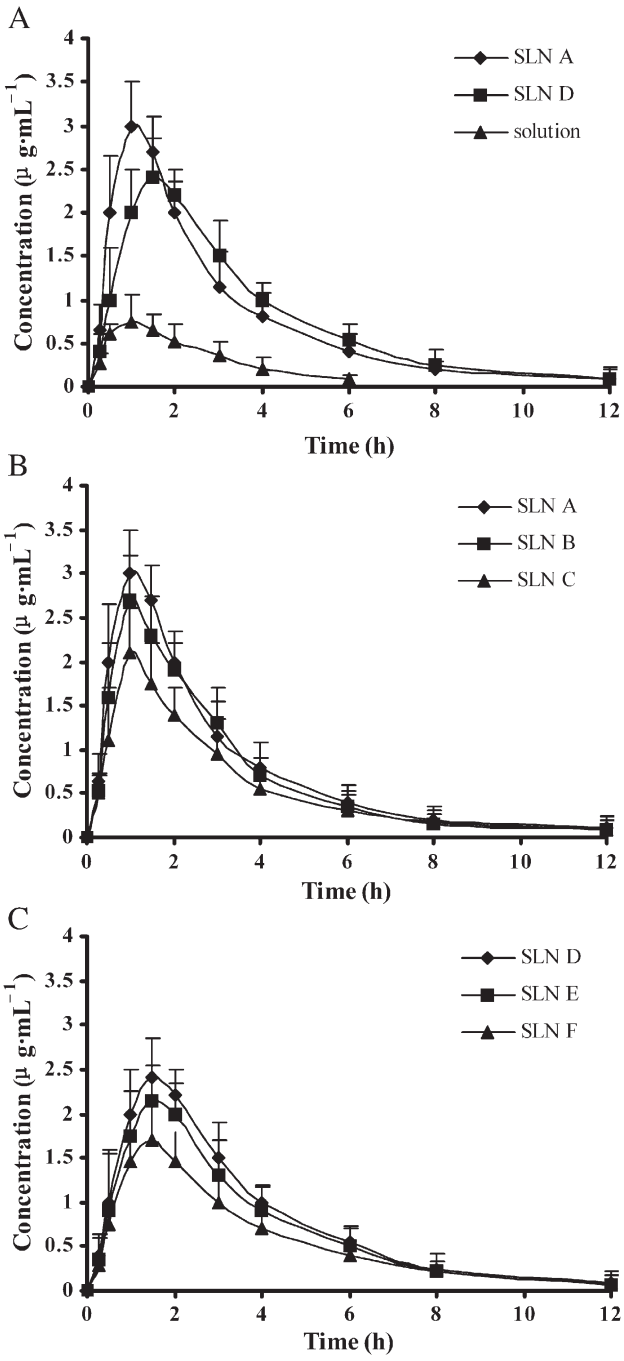

Fig. 4. The mean concentration–time curve after a single oral administration of different VIN formulations in rats (10 mg kg^{-1}). (A) SLN A, SLN D and VIN solution. (B) VIN-SLN containing different amounts of Tween 80. (C) VIN-SLN containing different amounts of Polyoxyethylene hydrogenated castor oil. Data are means±S.D., $n=6$.

Table 4
Pharmacokinetic parameters of VIN after oral administration of different formulations ($n=6$, $\bar{x}\pm s$)

Parameters	SLN A	SLN B	SLN C	SLN D	SLN E	SLN F	VIN solution
C_{max} (μg mL^{-1})	3.00±0.45 *	2.70±0.53 *	2.10±0.48 *	2.40±0.62 *	2.15±0.39 *	1.70±0.34 *	0.75±0.13
T_{max} (h)	0.92±0.21	0.97±0.17	1.03±0.19	1.33±0.23	1.45±0.26	1.51±0.31	1.01±0.20
K_e (h^{-1})	0.257±0.08 *	0.255±0.09 *	0.223±0.11 *	0.319±0.07	0.323±0.12	0.256±0.09 *	0.473±0.14
AUC (μg h mL^{-1})	9.56±1.33 *	8.67±1.52 *	6.98±1.08 *	9.58±1.19 *	8.57±0.83 *	7.04±1.76 *	2.30±1.22
MRT	3.42±0.47	3.39±0.52	3.88±0.39	3.65±0.58	3.66±0.44	4.14±0.49	2.59±0.32
CL (L h^{-1} kg^{-1})	1.04±0.12 *	1.15±0.23 *	1.43±0.09 *	1.04±0.18 *	1.17±0.28 *	1.42±0.21 *	4.35±0.34
V (L kg^{-1})	4.07±0.54 *	4.52±0.49 *	6.42±0.72	3.27±0.39 *	3.61±0.46 *	5.55±0.64	9.19±0.83
F_r	4.16±0.67 *	3.77±0.53 *	3.03±0.48 *	4.17±0.72 *	3.73±0.89 *	3.06±0.62 *	1

* $P < 0.05$ compared with VIN solution.

SLNs resulted in increased absorption of VIN. The $AUC_{0\to\infty}$ values of VIN after oral administration of SLN A and SLN D were 4.16- and 4.17-fold higher than those obtained with the VIN solution. From these results, we can conclude that VIN absorption was enhanced significantly by employing the SLN formulations compared with a VIN solution.

In the subsequent studies, the VIN-SLN containing different amounts of Polyoxyethylene hydrogenated castor oil (SLN D 2.0%, SLN E 1.5%, SLN F 1.0%) and Tween 80 (SLN A 2.0%, SLN B 1.5%, SLN C 1.0%) were employed to investigate the effect of surfactant on VIN absorption enhancement. Fig. 4B–C shows the plasma concentration vs time profiles following administration of these different formulations. The oral pharmacokinetic parameters are also given in Table 4.

On increasing the concentration of Polyoxyethylene hydrogenated castor oil from 1.0% to 1.5%, a significant absorption enhancement occurred. When Polyoxyethylene hydrogenated castor oil was further increased to 2.0%, the $AUC_{0\to\infty}$ also increased. The absorption was greatly improved by increasing the amount of Polyoxyethylene hydrogenated castor oil. On increasing the concentration of Tween 80 from 1.0% to 1.5%, the $AUC_{0\to\infty}$ increased from 6.98±1.08 μg mL^{-1} to 8.67±1.52 μg mL^{-1}, the absorption was greatly improved. When Tween 80 was further increased to 2.0%, there was also a significant difference either in the $AUC_{0\to\infty}$ or the C_{max} values of VIN compared with formulations containing 1.5% Tween 80.

Although many techniques have been employed to improve the oral absorption of poorly soluble drugs, little information is available in the literature on the improvement in the oral absorption of poorly soluble drugs by incorporation into SLNs. Our laboratory has carried out many studies on SLNs for solubilization and improving the oral absorption of poorly soluble drugs. The exciting results we obtained showed that VIN absorption was enhanced significantly by employing SLNs. It appears that SLNs offer a promising delivery system for enhancing the oral absorption of poorly soluble drugs. We will try to summarize the possible mechanisms by which the oral absorption of VIN is improved by SLNs.

Like other formulations, such as microemulsions or submicron emulsions, reduction in the particles size is a key factor for improving the peroral performance of poorly soluble drugs. In SLN formulations, the particle size range was reduced to less than 100 nm, resulting in an increase in surface area and saturation solubility. In-vitro release tests have confirmed that the release velocity from SLNs is significantly faster than the diffusion rate of VIN solution. An increase in saturation solubility and, consequently, an increase in the release rate of the drug allows it to reach high concentrations in the gastrointestinal tract. Drug absorption from the gastrointestinal tract (i.e. the intestine membrane transfer) may be depicted by a passive diffusion, where the driving force for diffusion across the membrane is the concentration gradient, so that a high local concentration can increase drug absorption. While considering the VIN solution, after preparation for about 1 h, microcrystals appeared in the solution with a relatively slow dissolution rate, so we predicted that the drug might precipitate at the gut wall after administration and thus result in a reduced oral absorption.

In our study, Tween 80 and Polyoxyethylene hydrogenated castor oil were used in the SLN formulations. Firstly, they were essential for producing SLNs of a small size and good physical stability. Secondly, in in-vivo studies, we found that they also played a key role in the oral absorption of VIN.

Due to their small particle size, SLNs may exhibit bioadhesion to the gastrointestinal tract wall or enter the intervillar spaces thus increasing their residence time in the gastrointestinal tract [14,15]. This increase in adhesion will result in enhanced bioavailability.

On increasing the amount of Tween 80 and Polyoxyethylene hydrogenated castor oil, it seems that there was not only a reduction in particle size, but also an improvement in the absorption of VIN. The surfactants may have contributed to an increase in the permeability of the intestinal membrane or improved the affinity between lipid particles and the intestinal membrane [16,17]. Some particles may be taken up into the lymphatic organs and eventually enter the systemic circulation [18–20]. A number of studies have reported an improvement in the oral absorption of poorly soluble drugs by co-administration of various P-glycoprotein inhibitors and cytochrome P450 (CYP) 3A inhibitors [21]. Tween 80 and Pluronic F-68 might moderately inhibit the P-glycoprotein efflux system [22,23], leading to the improved oral absorption of VIN.

Another advantage of SLN formulations over VIN solution is the lipid protection of the drug from chemicals, as well as enzymatic degradation, thereby delaying the in-vivo metabolism. Different CYP isozymes are able to catalyse the apovincaminic acid, the main pathway of VIN metabolism [24–26]. By incorporation into nanoparticles, VIN can be embedded into a solid lipid matrix thus reducing its exposure to enzymatic degradation following absorption.

From the statistical analysis of in-vivo pharmacokinetic data, we concluded that SLNs improved the bioavailability of VIN significantly compared with VIN solution and the amount of surfactant also significantly enhanced the oral absorption of VIN in SLNs.

Many reports have shown that the use of submicron emulsions was a promising way to improve oral drug absorption [27]. In our study, The aqueous solubility of VIN is almost nil (5.0 μg mL^{-1}) [28] and its solubility in lipophilic solvents, such as soybean oil, is also quite low and precludes the use of simple oil-in-water emulsion formulations. We made many attempts to increase its stability but the results were all unsatisfactory. In SLNs, no precipitation of VIN was observed after 1 year of storage.

The use of SLNs opens up new perspectives for the formulation of poorly soluble drugs. The poor aqueous solubility of VIN also makes it difficult to prepare parenteral formulations. To date, only a transfusion parenteral formulation is available for clinical use, but it needs multiple administrations. Some studies report that SLNs also have the potential for parenteral administration [8]. So, the SLN formulations cannot only improve the oral absorption of VIN, but can also be formulated for parenteral use, and further studies of this field are needed.

4. Conclusion

In our study, a poorly aqueous-soluble drug VIN was successfully incorporated into SLNs by an ultrasonic-solvent emulsification technique. The physicochemical characterization and long-term physical stability were investigated. The in-vitro release tests showed that the release velocity of the SLNs is always fast compared with the diffusion rate of the VIN solution. An oral pharmacokinetic study was conducted in male rats and the results showed that SLNs produced a significant improvement in the bioavailability of VIN compared with VIN solution. The amount of surfactant also had an important influence on the oral absorption of VIN. The absorption mechanisms of the SLN formulations are discussed. It appears that SLNs offer a promising delivery system for the enhancement of the bioavailability of poorly soluble drugs.

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