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Preparation and characterization of vinpocetine loaded nanostructured lipid carriers (NLC) for improved oral bioavailability

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ABSTRACT

The purpose of this study is to develop an optimized nanostructured lipid carriers (NLC) formulation for vinpocetine (VIN), and to estimate the potential of NLC as oral delivery system for poorly water-soluble drug. In this work, VIN–loaded NLC (VIN–NLC) was prepared by a high pressure homogenization method. The VIN–NLC showed spherical morphology with smooth surface under transmission electron microscope (TEM) and scanning electron microscopic (SEM) analysis. The average encapsulation efficiency was 94.9 ± 0.4%. The crystallization of drug in NLC was investigated by powder X-ray diffraction and differential scanning calorimetry (DSC). The drug was in an amorphous state in the NLC matrix. In the in vitro release study, VIN–NLC showed a sustained release profile of VIN and no obviously burst release was observed. The oral bioavailability study of VIN was carried out using Wistar rats. The relative bioavailability of VIN–NLC was 322% compared with VIN suspension. In conclusion, the NLC formulation remarkably improved the oral bioavailability of VIN and demonstrated a promising perspective for oral delivery of poorly water-soluble drugs.

1. Introduction

Lipid-based drug delivery systems are expected as promising oral carriers because of their potential to increase the solubility and improve oral bioavailability of poorly water-soluble and/or lipophilic drugs (O'Driscoll and Griffin, 2008). Traditional lipid-based formulations include a broad range of lipid solution, emulsions, liposomes, lipid microparticles and nanoparticles. Among the formulations above, the nanostructure lipid carriers (NLCs) are regarded as the second-generation of lipid nanoparticles (Müller et al., 2002), and are attracting major attention as alternative colloidal drug carriers. NLC developed from solid lipid nanoparticles (SLN) are composed of biocompatible solid lipid matrices and liquid lipid which is considerably different in chemical structure from solid lipid. NLC system possesses many advantages of SLN, such as good biocompatibility, controlled drug release, and the possibility of production on large industrial scale. However, the various kinds of lipid components of NLC results in the structure with imperfections type, amorphous state type or multiple type to accommodate more drug and reduce the drug leakage during storage so as to overcome potential limitations associated with SLN (Müller et al., 2002). The lipid formulations loaded with poorly water-soluble drugs for oral route have been investigated and reported to improve the oral bioavailability by many research teams (Shah et al., 1994; Mohamed et al., 1998; Paliwal et al., 2009), but there are few reports on NLC system for oral administration yet. In this study, we made efforts to make investigations in this research field.

Vinpocetine (VIN), a vincamine derivative, is now widely used for the treatment of chronic cerebral vascular ischemia, acute stroke, senile cerebral dysfunction and Alzheimer's disease (Chiu et al., 1988; Bönöczk et al., 2000). However, VIN is a poorly water-soluble drug (the water solubility ≈5 μg/ml) with a short half-life time of about 2 h, and is cleared by extensive metabolism in the liver (Polgár et al., 1985; Miskolczi et al., 1990). Due to the slow dissolution rate in the intestinal tract and significant first-pass effect, the oral bioavailability of VIN in human is as low as 7% which largely restricts its clinical use (Szakács et al., 2001). Therefore, to overcome these difficulties, it is necessary to design a formulation possible to improve the oral absorption and bioavailability of VIN.

The objective of the present study is to design and assess a NLC delivery system as a potential oral formulation of VIN. Firstly, the VIN–loaded NLCs were developed and screened, and the physico-

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Table 1
Composition of different NLC formulations (%, w/v).

	VIN	Compritol 888 ATO	Monostearin	Miglyol 812N	Lecithin	Solutol HS-15	Poloxamer 188	Water
NLC-1	0.1	4		1	1.2	2		91.7
NLC-2	0.1	4		1	1.2		2	91.7
NLC-3	0.1		4	1	1.2	2		91.7
NLC-4	0.1		4	1	1.2		2	91.7

chemical characteristics of which were investigated in detail. Then, the formulation of NLC was evaluated on the drug release properties in vitro and oral bioavailability in vivo. Finally, the possible absorption mechanisms of NLC formulations were discussed.

2. Materials and methods

2.1. Materials

Vinpocetine was provided by Zhengzhou Lingrui Parmaceutical Co., Ltd. (Henan, China), Compritol 888 ATO (glyceryl behenate) was obtained as a gift from Gattefosse (France), Lecithin (S75) was purchased from Lipoid (Germany), Solutol HS-15 (polyoxyethylene esters of 12-hydroxystearic acid) and Poloxamer 188 (polyethylene-polypropylene glycol) was kindly supplied by BASF (Germany), Miglyol 812N (C_8–C_{12} triglyceride) was gifted by Sasol (Germany), monostearin (glycerol 1-monostearate) was purchased from Shanghai Chemical Co. (Shanghai, China). All other chemicals and reagents used were at least of analytical grade.

2.2. Preparation of VIN–loaded NLCs

The VIN–loaded NLCs were prepared by a high pressure homogenization method. Table 1 shows the four formulations investigated. Briefly, the desired amounts of VIN, the solid lipid (Compritol 888 ATO or monostearin) and Miglyol 812N together were blended and melted at 85 °C to form a uniform and clear oil phase. Meanwhile, the aqueous phase was prepared by dispersing surfactant (Solutol HS-15 or Poloxamer 188) and lecithin in double-distilled water and heated to the same temperature. Then the hot aqueous phase was added drop wisely to the oil phase at 85 °C under magnetic stirring at 600 rpm. The coarse emulsion was obtained by using a high shearing force emulsifier (IKA Co., Guangzhou, China) at 10,000 rpm for 5 min. And then the final dispersion was produced by passing the hot coarse emulsion three circles through a high pressure homogenizer (NS1001L, Niro, Italy) at pressure of 800 bar and temperature of about 70 °C. Subsequently the dispersion was cooled to room temperature to solidify nanoparticles. The formulations were stored at 4 °C.

2.3. HPLC analysis of VIN

The content of VIN in various mediums was analyzed using reverse-phase HPLC methods. The HPLC system was composed of a model LC-10AT pump (Shimadzu, Kyoto, Japan) and a model SPD-10A UV detector (Shimadzu, Kyoto, Japan). The analytical column was Diamonsil C18 (200 × 4.6 mm, 5 μm) (Dikma, USA). The injection volume was 20 μl; the mobile phase was methanol–0.1 mol/l ammonium carbonate aqueous solution (90/10, v/v) at a flow rate of 1.0 ml/min; the UV detector wavelength was 273 nm; the column temperature was 30 °C.

2.4. Drug encapsulation efficiency and drug loading

Encapsulation efficiency (EE) of VIN–NLCs was calculated by determining the amount of free drug using ultrafiltration technique. 1 ml VIN–loaded NLC colloidal solution was placed in the upper chamber of a centrifuge tube matched with an ultrafilter (Amicon ultra, Millipore Co., USA, MWCO 10 kDa) and centrifuged for 10 min at 4000 rpm. The ultrafiltrate containing the unencapsulated drug was determined by HPLC. The total drug content in VIN–NLC was determined as follows: aliquots of 1 ml VIN–NLC dispersion were dissolved and diluted appropriately by methanol to dissolve the NLC and then the obtained suspension was allowed to filter through 0.45 μm membrane filters. The resulting solution was analyzed by HPLC. The drug loading content was the ratio of incorporated drug to lipid (w/w). The EE and drug loading could be calculated by the following equations:

$$\text{EE\%} = \frac{W_{\text{Total}} - W_{\text{Free}}}{W_{\text{Total}}} \times 100$$

$$\text{Drug loading(\%)} = \frac{W_{\text{Total}} - W_{\text{Free}}}{W_{\text{Lipid}}} \times 100$$

where W_{Total}, W_{Free}, W_{Lipid} were the weight of total drug in NLC, the weight of unentrapped drug in ultrafiltrate and the weight of lipid added in system, respectively.

2.5. Particle size and zeta potential

The average particle size and size distribution of VIN–NLCs were determined by Laser Particle Size Analyzer (Coulter LS-230, Beckman Coulter Co. Ltd., USA) at room temperature. The particle size analysis data obtained were evaluated using the volume distribution. The zeta potential was measured using a zeta potential analyzer (Delsa 440 SX, Beckman Coulter Co. Ltd., USA). The samples were allowed to dilute with double-distilled water prior to zeta potential determination.

2.6. TEM and SEM analysis

The morphology of VIN–NLC colloidal solution was observed by transmission electron microscopy (JEM-1200EX, JEOL). The sample demanded was prepared by placing a drop of VIN–NLC which was diluted 50-fold with double-distilled water onto a 400-mesh copper grid coated with carbon film and followed by negative staining with 1% phosphotungstic acid. Then the sample was dried in the air before TEM observation.

The surface morphology of VIN–loaded NLC was visualized by scanning electron microscopy (SSX-550, Shimadzu, Kyoto, Japan). Before observation, the lyophilized nanoparticles were fixed on a double-sided sticky tape which had previously been secured on aluminum stubs and then coated with gold in an argon atmosphere. The samples were imaged using SSX-550 with an accelerating voltage of 5–10 kV.

2.7. Thermal analysis

Differential scanning calorimetry (DSC) analysis was performed using a DSC-60 differential scanning calorimeter (Shimadzu, Japan). The samples weighed accurately were placed in aluminum pans and sealed with a lid. Al_2O_3 was used as the reference. In the scanning process, a heating rate of 5 °C was applied in the temperature range from 20 to 250 °C with a nitrogen purge of 0.2 ml/min.

2.8. Powder X-ray diffraction analysis

Powder X-ray diffraction (PXRD) was used to identify the crystal form of VIN dispersed in the matrix of lipid. PXRD studies were performed by powder X-ray diffractometer (X'PERT PRO SUPER, Panalytica, Holland) using Cu Kα radiation. The samples were scanned over a 2θ range of 5–50° at a scan rate of 0.05°/s. Samples used for measurement were pure VIN, Compritol 888 ATO, physical mixture, freeze-dried VIN–NLC, respectively.

2.9. In vitro release studies

The vitro release studies of VIN from NLC were carried out by the bulk-equilibrium reverse dialysis technique (Levy and Benita, 1990; Washington, 1990). The release medium was 0.1 M HCl, phosphate-buffered saline (PBS) pH 6.8 at 37 °C, respectively. And the stirring speed was set at 50 rpm. As previously reported (Levy and Benita, 1990), dialysis bags (molecular weight cut off 12–14 kDa) containing aliquots of 2 ml of sink solution were equilibrated in release medium for 12 h prior to experiment. Aliquots of 1 ml of VIN–loaded NLC were directly placed into 900 ml of release medium. At intervals, one dialysis bag was withdrawn from the release solution and the same volume of fresh medium was added to maintain a constant volume. Then the release media in the dialysis bags was analyzed by HPLC.

2.10. In vivo study

All animal experiments were carried out according to the Principles of Laboratory Animal Care (People's Republic of China). And the protocols for the animal studies were approved by the Department of Laboratory Animal Research at Shenyang Pharmaceutical University. Male Wistar rats weighing 200–250 g were obtained from Animal experimental center of Shenyang Pharmaceutical University and housed at temperature of 22 ± 2 °C and 45–50% relative humidity. All the rats were divided randomly into two groups comprising six animals in each and fasted overnight but allowed to free access to water before experiment.

The rats were given VIN–NLC (1 mg/ml) or an equivalent dose of VIN suspension, respectively. The VIN–NLC formulation used was NLC-1 (Compritol 888 ATO as solid matrix and Solutol HS-15 as surfactant). The VIN suspension was prepared by dissolving commercial VIN tablets in a 0.5% CMC-Na solution and sonicated for 10 min. Blood samples (0.5 ml) of each animal were sampled via the suborbital vein at 0, 0.083, 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24 h after administration. All the blood samples were immediately centrifuged at 4000 × *g* for 10 min to separate the plasma. The plasma obtained was stored at −20 °C until analysis.

The concentrations of VIN in rat plasma were determined by HPLC (Elbary et al., 2002). The HPLC system was composed of a model LC-10AT pump (Shimadzu, Kyoto, Japan) and a model SPD-10A UV detector (Shimadzu, Kyoto, Japan). The analytical column was Diamonsil C18 (200 × 4.6 mm, 5 μm) (Dikma, USA). The injection volume was 20 μl; the mobile phase was methanol–water–glacial acetic acid (70/30/0.1, v/v/v); the flow rate was 1.0 ml/min; the UV detector wavelength was 273 nm; the column temperature was 35 °C.

Plasma samples were processed as follows: a 200 μl plasma sample was mixed with 20 μl of an internal standard (diazepam) solution (2 μg/ml) and 100 μl 0.5 M NaOH and vortexed for 1 min. Then 2 ml of ether anhydrous was added and the mixture was vortexed at room temperature for 5 min. After centrifugation at 3000 × *g* for 10 min, the organic layer was transferred to a new tube and evaporated under a stream of high purity nitrogen at 40 °C. The residue was reconstituted with 100 μl of mobile phase by vortexing for 5 min and 20 μl was analyzed by HPLC.

Table 2
The average particle size, zeta potential and EE of the four VIN–NLC formulations, each value represents the mean ± S.D. ($n = 3$).

	Average size (nm)	Zeta potential (mV)	EE (%)
NLC-1	136 ± 4.2	−13.4 ± 2.0	95.3 ± 1.4
NLC-2	177 ± 5.4	−24.7 ± 1.4	94.6 ± 1.8
NLC-3	107 ± 8.7	−14.5 ± 1.1	86.4 ± 3.1
NLC-4	132 ± 6.1	−23.5 ± 0.7	83.2 ± 2.3

The main pharmacokinetic parameters were acquired with the help of a pharmacokinetic program DAS 2.0. The values of maximum concentration (C_{max}) and time of maximum concentration (T_{max}) were obtained directly from the concentration–time plotting. The area under the concentration–time curve (AUC) was calculated by linear trapezoidal method. The relative bioavailability of NLC formulations was calculated using the following formula:

$$\mathrm{Fr}(\%) = \frac{\mathrm{AUC}_T D_R}{\mathrm{AUC}_R D_T}$$

where Fr was the relative bioavailability, AUC was the area under the plasma concentration–time curve, D was the dose administrated, T was the test formulation (oral administration of VIN–loaded NLC colloidal solution), R was the reference formulation (oral administration of VIN suspension).

2.11. Statistical analysis

All values obtained were expressed as mean ± standard error mean (S.E.M.). Statistical comparisons were performed by analysis of variance and Student's t-test using SAS Version 8.0 software. A value of $p \leq 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Preparation and characteristic of VIN–NLC

In this study, Compritol 888 ATO (glyceryl behenate) or monostearin (glycerol 1-monostearate) was chosen for the solid lipid matrix, and Miglyol 812N, a C_8–C_{12} triglyceride, was used as the liquid lipid of the matrix which made the NLC different from the formulation of solid lipid nanoparticles (SLN). The non-toxic, non-ionic surfactant, Solutol HS-15 (polyoxyethylene esters of 12-hydroxystearic acid) or Poloxamer 188 (polyethylene–polypropylene glycol) was used in combination with lecithin as surfactant.

NLCs can be prepared by various methods including hot and cold homogenization, solvent diffusion and microemulsion (Labouret et al., 1995; Cavalli et al., 1998; Mühlen and Mehnert, 1998; Mühlen et al., 1998; Trotta et al., 2003; Hu et al., 2005). The hot high pressure homogenization method was simple and quick on lab scale. In this study, the average particle size and zeta potential of four different VIN–NLC formulations were shown in Table 2. The EE of the NLC formulations using Compritol 888 ATO as solid matrix were obviously higher than that using monostearin. On the other hand, the combination of Solutol HS 15 and lecithin as surfactants might slightly increase the encapsulation efficiency compared with Poloxamer 188. The major reason might be that VIN had higher solubility in Compritol 888 ATO than in monostearin. Secondly, Solutol HS 15 was recommended as non-ionic solubilizing agent to be added to injection formulations (Reinhart and Bauer, 1995; Ruchatz and Schuch, 1998). In the formulation of NLC-1, the lipophilic segments (carbon chain of fatty acid) of Solutol HS 15 partially inserted into lipid core whereas the hydrophilic PEG chains protruded towards the external water phase to form stereospecific blockade. Because of the high HLB value of Solutol HS 15, lecithin was added to the

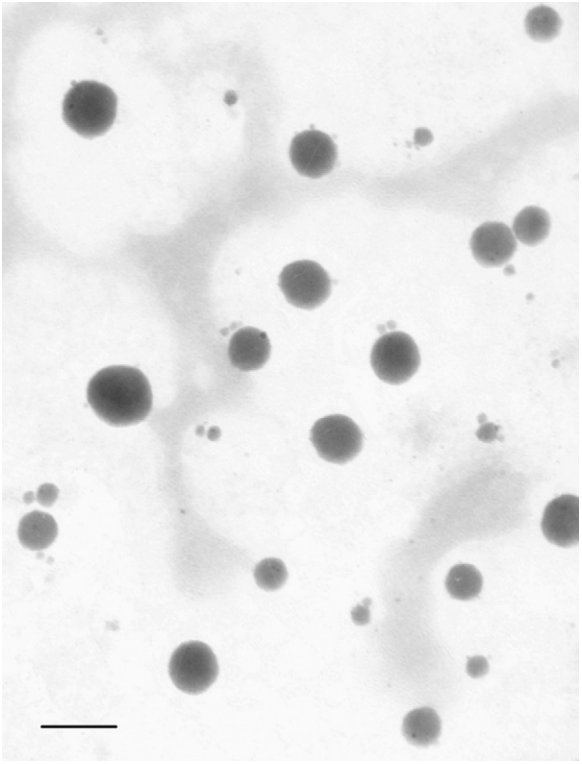

Fig. 1. TEM morphology of VIN–NLC. Bar = 200 nm.

system to adjust the HLB of co-surfactants so as to enhance the ability to emulsify the lipid and stability of the system. The lecithin was mainly distributed at the interface of the oil and the aqueous phase. Meanwhile, Compritol 888 ATO and Miglyol 812N exhibited good miscibility in the nanoparticulate state (Volkhard et al., 2000).

The mean diameters of the NLC formulations containing monostearin were 107 ± 8.7 and 132 ± 6.1 nm comparing with that 136 ± 4.2 and 177 ± 5.4 nm of Compritol 888 ATO. The Compritol 888 ATO had higher melting point and viscosity, so it is more difficult to obtain small and uniform size distribution. Taking all the factors into consideration, the Solutol HS 15 and soybean lecithin were chosen as surfactants.

The zeta potential of formulations including Solutol HS 15 was higher than that of Poloxamer 188. The PEG chains of Solutol HS 15 protruded towards the external water phase to form stereospecific blockade which blocked out ions from the surface, so the zeta potential was higher (García-Fuentes et al., 2002).

The average drug loading of the four VIN–NLCs were $1.80 \pm 0.12\%$.

In conclusion, the VIN–NLC combining Compritol 888 ATO with Solutol HS 15 had good ability to encapsulate drug and displayed favorable physicochemical characteristics.

3.2. Morphology of VIN–NLC

The morphology of VIN–NLC determined by TEM was shown in Fig. 1. The TEM study demonstrated that the particles had almost spherical and uniform shapes and did not stick to each other. The mean diameter was in the range of 100–150 nm.

Fig. 2 shows the image of surface morphology and internal structure of VIN–NLC after freeze-drying. The results indicated that the particles were round and homogeneous with smooth surface and fixed in the bulk and grid structure formed by cryoprotectants. There was no drug crystal or aggregation of particles visible in the graph. The lyophilized powder could be re-dispersed in water easily. This would be helpful for the reconstitution of the dry powder and stability during storage.

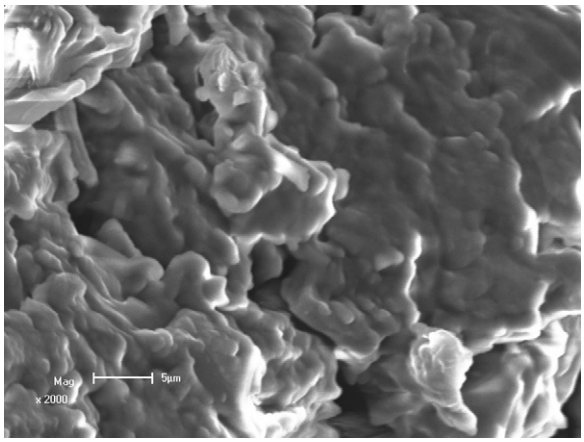

Fig. 2. SEM morphology of VIN–NLC, showing the surface structure of the lyophilized powder of VIN–NLC. Bar = 5 μm.

3.3. The crystal form of VIN–NLC

DSC was a basic method to investigate the crystallization and state of drug in the compounds and NLC by determining the variation of temperature and energy at phase transition. Fig. 3 shows DSC curve of Compritol 888 ATO, VIN, their physical mixture and VIN–NLC lyophilized powder. The thermogram of VIN showed a melting peak of VIN at around 153 °C. For Compritol 888 ATO, the melting process took place with maximum peak at 74 °C. These melting peaks all appeared and had almost same value in the curve of the physical mixture. However, the melting peak for the VIN around 153 °C was not detected in the thermograms of the lyophilized VIN–NLC. The disappearance of the endothermic peak of VIN–NLC powder suggested that VIN in lipid was not in crystalline state but was in amorphous state to form solid solution within the matrix of nanoparticles.

In order to identify the physical state of VIN incorporated in NLC, powder X-ray diffraction (PXRD) was used and the patterns of Compritol 888 ATO, pure VIN, their physical mixture and lyophilized VIN–loaded NLC were shown in Fig. 4. In the PXRD diagram of pure VIN, significant diffraction peaks which indicated crystalline nature of VIN can be detected at 2θ scattered angles 11°, 12°, 14°, 15°, 17.5°, 19°, 23°, 24°, 29° and 31.5°. The pattern of physical mixture showed two characteristic wide peaks (21° and 24°) of Compritol 888 ATO

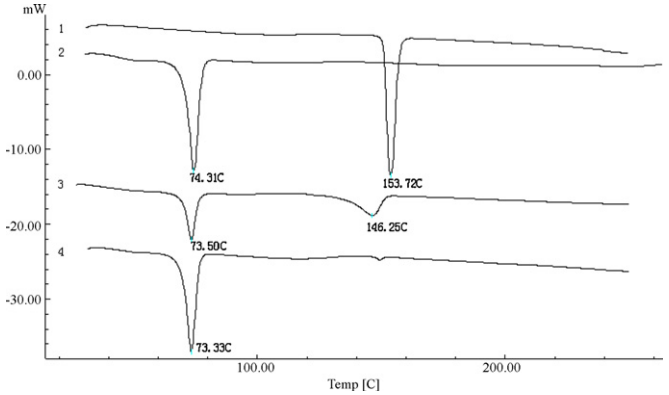

Fig. 3. DSC image of VIN–NLC and its ingredients. 1: VIN; 2: Compritol 888 ATO; 3: physical mixture of VIN and Compritol 888 ATO; 4: VIN–NLC.

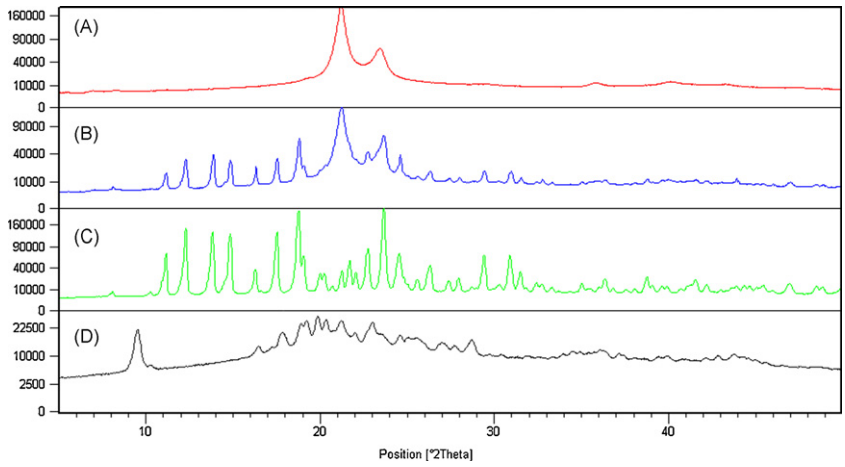

Fig. 4. Powder X-ray diffraction image of VIN–NLC and its ingredients. A: Compritol 888 ATO; B: physical mixture of VIN and Compritol 888 ATO; C: VIN; D: VIN–NLC.

and decreased peak intensities of pure VIN. It indicates that the bulk matrix (Compritol 888 ATO) was in good crystal. To the contrary, no sharp characteristic peak for VIN crystalline was observed in the VIN–NLC powder. In the pattern of VIN–NLC, the broad predominated but small peaks at 2θ scattered angles 9.3°, 20.4° and 24° can possibly be attributed to the crystalline structure of Compritol 888 ATO and the cryoprotectants. The results suggest that VIN was in molecular state not in crystalline form in NLC.

3.4. In vitro release study

In this study, the bulk-equilibrium reverse dialysis technique was chosen to investigate VIN release from VIN–NLC and VIN tablet in different medium. This method is quite different from conventional dialysis bag diffusion which is widely used in measuring the drug release of colloidal solution. In dialysis diffusion method, the sample is added in the dialysis bag and is not diluted with enough medium; the diffusion process of drug was slow because of the low concentration grade and the barrier of dialysis. The dialysis diffusion method does not simulate the true release profile of drug in vivo. However, in the bulk-equilibrium reverse dialysis method, the VIN–loaded NLC colloidal solution was directly placed in the release solution where it has the opportunity to release the drug under maximum dilution (perfect sink conditions) (Levy and Benita, 1990). The nanoparticles were directly exposed to large volume of sink release medium and better diluted. Therefore, the diffusion rate of drug from nanoparticles to sink solution was high. The dialysis in reverse can truly reflect the release of drug in a natural biological environment in vivo.

The VIN release profiles in 0.1 M HCl and pH 6.8 PBS were shown in Figs. 5 and 6, respectively. Comparing of the release curves of VIN–NLC in pH 1.2 and pH 6.8, we concluded that VIN–NLC showed no burst release at initial stage in both medium, which was evidence that there was no unencapsulated drug attaching to the surface of the particles. A sustain release profile was demonstrated in pH 6.8. The percent of accumulated dissolution of VIN–NLC in PBS 6.8 over 48 h was less than 20%, which was less than 5% of VIN in commercial tablets. However, VIN commercial tablets in 0.1 M HCl tended to release a high amount of drug (up to 90%) at 4 h because of the faintly alkaline of VIN while that was less than 10% in VIN–NLC. The results suggest that the VIN was entrapped in NLC and was protected from the strong acidic environment of the stomach and then the VIN–NLC subsequently reached small intestinal. The results suggested that the major content of VIN in NLC could be uptaken by intestinal cells and enter the body circulation to perform sustained release in vivo.

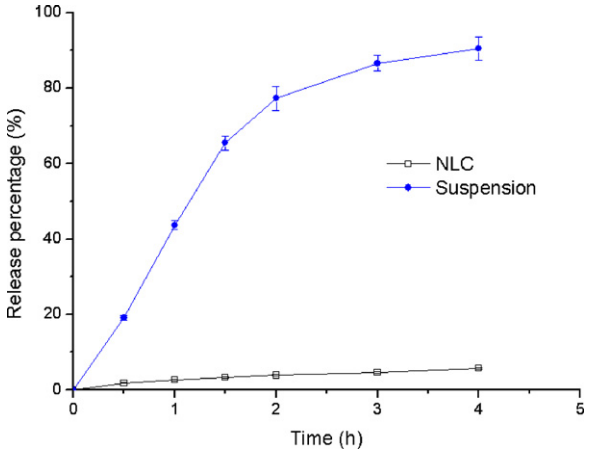

Fig. 5. In vitro release profile of VIN from different vehicles at pH 1.2. Each value represents the mean ± S.D. ($n = 3$).

The release profile of drug from NLC in pH 6.8 PBS was fitted to a Ritger–Peppas kinetics model and the equation was as follows: $\ln R = 0.8121 \ln t - 0.2352$ ($r = 0.9976$). Based on the fitting result of Ritger–Peppas model ($\ln R = k \ln t + C$) (Ritger and Peppas, 1987), the value of k was 0.8121 ($0.45 < k < 0.85$), it suggests that VIN release from NLC was the mixing of drug diffusion and lipid matrix erosion. The k value was close to 0.85, which indicated that the effect of

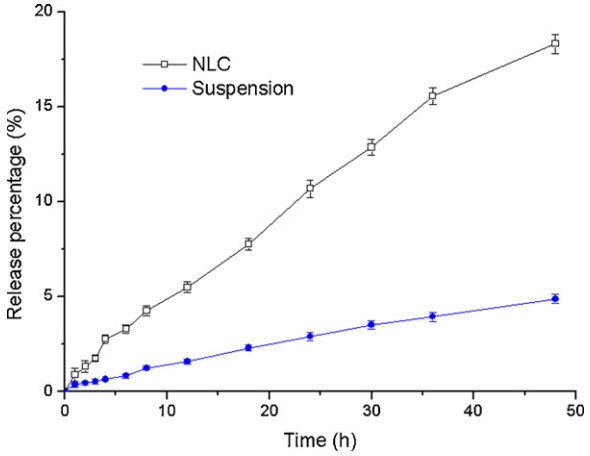

Fig. 6. In vitro release profile of VIN from different vehicles at pH 6.8. Each value represents the mean ± S.D. ($n = 3$).

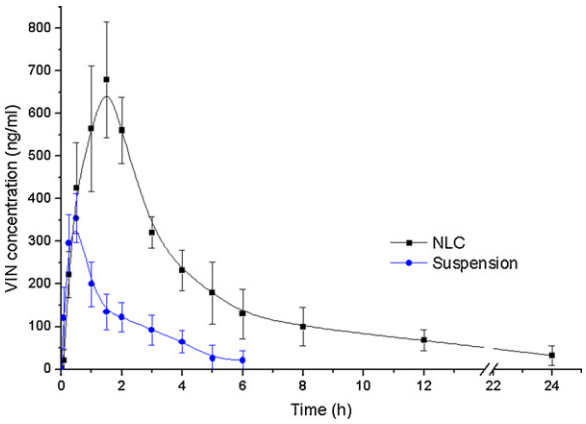

Fig. 7. Plasma concentration vs. time plotting of VIN after oral administration in rats. Each value represents the mean ± S.D. ($n = 6$).

matrix erosion is predominant and the major amount of drug is enriched in the core of NLC. The miner amount of drug in the shell can diffuse into the medium.

3.5. In vivo studies

VIN–NLC-1 (Compritol 888 ATO and Solutol HS 15) and VIN suspension were orally administrated to Male Wistar rats. The plasma concentration–time plots in rats after oral administration were shown in Fig. 7. The T_{max} was 0.5 h and the C_{max} value was 354.29 ± 57.49 ng/ml after oral administration of VIN suspension. However, the T_{max} value (1.5 h) of VIN–NLC was an hour later than that of VIN suspension. The visible difference between T_{max} value of VIN–NLC and VIN suspension manifested that the rates of absorption of two formulations were not the same. VIN in suspension dissolved in the intestinal tract and absorbed directly into systemic circulation. Therefore, the VIN reached the peak concentration quickly at 0.5 h. However, VIN in NLC could hardly be released into the gastrointestinal tract, as was supported in the in vitro release studies. Therefore, the intact VIN–NLCs were directly absorbed into the blood circulation and released the drug gradually. The C_{max} value of VIN–NLC was 679.29 ± 135.57 ng/ml which was significant higher than that obtained with the VIN suspension (354.29 ± 57.49 ng/ml). Meanwhile, at all time points except the first three, the VIN plasma concentrations in rats treated with VIN–NLC were remarkably higher than those treated with VIN suspension. Twenty-four hours after oral administration of VIN–NLCs, the VIN plasma concentrations was still 32 ng/ml, whereas the drug was undetectable 8 h after treated with VIN suspension. The corresponding pharmacokinetic parameters were listed in Table 3. The $AUC_{0\to t}$ after oral administration of VIN–NLC was 3143.9 ± 574.9 ng/ml/h, which was approximately 3.2-fold higher than that of VIN suspension (975.8 ± 109.4 ng/ml/h). The results indicated that systemic absorption of VIN was significantly enhanced by incorporating into NLC compared with VIN suspension. The NLCs showed a promising potential for enhancing oral bioavailability of poorly water-soluble drugs.

Table 3
Pharmacokinetics parameters of VIN in rat plasma after oral administration, each value represents the mean ± S.D. ($n = 6$).

Parameters	NLC	Suspension
C_{max} (ng/ml)	679 ± 136	354 ± 57*
T_{max} (h)	1.58 ± 0.20	0.42 ± 0.13*
$AUC_{0\to t}$ (ng/ml/h)	3144 ± 575	976 ± 109*
MRT (h)	5.65 ± 1.54	2.02 ± 0.29*
$K_e \times 10^3$ (h^{-1})	0.19 ± 0.06	0.52 ± 0.10*

* $p < 0.001$, comparison with NLC.

The possible reasons for low bioavailability of VIN were its poor water-solubility, extensive metabolism in the liver, and the barrier of the single layer of intestinal epithelial cell (Chen et al., 2008). Therefore, we summarized the possible mechanism of the improved oral bioavailability of VIN by employing NLC formulation. Firstly, NLC was composed of solid and liquid lipids which were structurally similar to fat rich in food. The lipids could induce bile secretion in the small intestinal and the VIN–loaded NLCs were associated with bile salt to form mixed micelles which helped the intact NLCs get into the lymphatic vessels and avoid the liver first-pass metabolism (Plain and Wilson, 1984; Yang et al., 1999; Jacobs et al., 2000). The uptake and lymphatic transport of intact NLCs played a dominant role in the promoted absorption.

Secondly, the particle size range of NLC formulation was almost less than 200 nm, and the reduction in particle size resulted in a great increase in surface area of the particles. Meanwhile, the high dispersibility of NLC was positive to a sufficient and steady absorption in the intestinal tract. In addition, the particles exhibited bioadhesion to the gut wall and the residence time was prolonged, which possibly resulted in enhanced oral absorption.

Based on the structure of VIN, it is a weak alkaline compound which brings a higher solubility at low pH value (1.2). After oral administration of VIN suspensions, the drug would firstly dissolve in the gastric fluid. Afterwards, when the drug reached the intestinal tract, the pH value increased significantly and resulted in precipitation of the drug, which affected the further absorption. On the other hand, VIN which was directly absorbed into the blood circulation would meet extensive metabolism of the liver, that is, the first-pass effect. In the case of VIN–NLC, first-pass effect could be largely avoided because that the drug was encapsulated in nanoparticles and they were available for the lymphatic absorbing pathway. The sustained release property of NLC could also achieve a longer retention time in vivo.

Another reason of the improved absorption might be attributed to the use of surfactant Solutol HS 15 in the NLC formulations. Surfactants might increase the intestinal epithelial permeability by disturbing the cell membrane and reversibly open the tight junction of intestinal epithelial cell (Lindmark et al., 1995). Solutol HS 15 has been discovered to have an affinity to the *p*-glycoprotein and it might be able to inhibit *p*-glycoprotein efflux pump and increase VIN transport across the intestinal mucosa (Morazzoni et al., 1993; Buszello et al., 2000).

4. Conclusion

In this study, VIN–NLC for oral administration was successfully prepared by a high pressure homogenization method. The VIN–NLC obtained showed small and homogeneous particle size with high encapsulation efficiency. In vitro release study indicated that VIN–NLC showed a sustained release profile of VIN without obvious burst-release effect. In vivo pharmacokinetic study showed that the relative bioavailability of VIN–NLC formulation was 322% compared with VIN suspension in Wistar rats after oral administration. The NLCs could improve the gastrointestinal absorption of VIN. The nanostructured lipid carrier offered a potential approach to enhance oral bioavailability of poorly water-soluble drug. Based on these findings, we will further study on the precise and specific mechanisms of improvement in oral absorption by this formulation.

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