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Isotretinoin-loaded solid lipid nanoparticles with skin targeting for topical delivery

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Abstract

The purpose of this study was to construct isotretinoin-loaded SLN (IT-SLN) formulation with skin targeting for topical delivery of isotretinoin. PRECIROL ATO 5 was selected as the lipid of SLN. Tween 80 and soybean lecithin were used as the surfactants to stabilize SLN. The hot homogenization method was performed to prepare the drug-loaded SLN. The various formulations were characterized by photon correlation spectroscopy and all the SLN formulations had low average size between 30 and 50 nm. Transmission electron microscopy studies showed that the IT-SLN formulation had a spherical shape. All the formulations had high entrapment efficiency ranging from 80% to 100%. The penetration of isotretinoin from the IT-SLN formulations through skins and into skins were evaluated in vitro using Franz diffusion cells fitted with rat skins. The in vitro permeation data showed that all the IT-SLN formulations can avoid the systemic uptake of isotretinoin in skins, however the control tincture had a permeation rate of $0.76 \pm 0.30\ \mu g\ cm^{-2}\ h^{-1}$ through skins. The IT-SLN consisting of 3.0% PRECIROL ATO 5, 4.0% soybean lecithin and 4.5% Tween 80 could significantly increased the accumulative uptake of isotretinoin in skin and showed a significantly enhanced skin targeting effect. The studied IT-SLN showed a good stability. These results indicate that the studied IT-SLN formulation with skin targeting may be a promising carrier for topical delivery of isotretinoin.

Keywords: Isotretinoin; Solid lipid nanoparticles; Skin targeting; Topical delivery

1. Introduction

Isotretinoin, a derivative of retinoic acid (13-*cis*-retinoic acid), has been commonly used for the treatment of severe acne and the other dermatological diseases (Katsambas and Papakonstantinou, 2004). However, it has obvious adverse side effects by oral administration. The launched topical preparations such as cream also show systemic absorption and significant skin irritation (Queille-Roussel et al., 2001). So, it is necessary to improve the skin uptake and reduce systemic absorption of isotretinoin using a carrier with an ability of skin targeting.

During the past several years, solid lipid nanoparticles (SLN) began to act as a topical carrier not only for pharmaceutical molecules, but also for cosmetic products (Müller et al., 2002). Compared with conventional carriers such as cream, tincture and emulsion, SLN combine their advantages such as controlled release, in vivo good toleration and protection of active compounds (Müller et al., 2000; Sylvia et al., 2003). Especially, SLN can favor drug penetration into the skins (Jenning et al., 2000; Wissing and Müller, 2003), maintain a sustained release to avoid systemic absorption (zur Mühlen et al., 1998), act as a UV sunscreen system (Wissing and Müller, 2002), reduce irritation (Maia et al., 2000; Sivaramakrishnan et al., 2004).

Recently, the research activities on SLN has gradually focused on the cosmetic and topical product, SLN as a topical carrier were used for topical delivery of several drugs including clotrimazole, prednicarbate and betamethasone 17-valerate and SLN was reported to have a skin targeting potential (Maia et al., 2000; Sivaramakrishnan et al., 2004; Souto et al., 2004; Song and Liu, 2005). SLN was found to have a skin targeting which can result in a high accumulation of podophyllotoxin in skin (Chen et al., 2006). The skin targeting of SLN for topical delivery aroused our interest. In this work, SLN was used for topical delivery of isotretinoin and the long-term aim is to explore a novel formu-

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lation with skin targeting effect for the treatment of severe acne. The present study focused on the preparation, characterization and skin targeting evaluation of the isotretinoin-loaded SLN (IT-SLN).

2. Materials and methods

2.1. Materials

PRECIROL ATO 5 was purchased from GATTEFOSSE (France). Soybean lecithin® was provided by Shanghai Taiwei Pharmacy Corp. (Shanghai, China). Tween 80 was obtained from Shanghai Chemical Reagent Corp. (China). Isotretinoin was obtained from Taizhou Aolite Minute Chemical Corp. (Zhejiang, China). Other chemicals are of HPLC or analytical grade.

2.2. Preparation of SLN

The IT-SLN formulations (Table 1) were prepared using hot homogenization method. PRECIROL ATO 5 (PA5), soybean lecithin (SL) and isotretinoin were dissolved in ethanol. Then, ethanol as the organic solvent was completely removed using rotoevaporator at 80 °C. The melt residue was added to the hot water containing Tween 80. Then, the pre-emulsion was prepared using a high speed stirrer (Model 1001, Shanghai Weiyu Corp., China) for 20 min at 6000 rpm. The pre-emulsion was processed at 800 bar, 80 °C for five cycles using a high pressure homogenizer (APV 2000, Denmark). After the homogenized sample was cooled at 5 °C, the SLN dispersions stabilized by Tween 80 and soybean lecithin was obtained. The tincture containing 0.06% isotretinoin as a control was prepared by dissolving 0.06 g isotretinoin into 99.94 g of 95% ethanol.

2.3. Characterization of SLN

2.3.1. Particle size and zeta potentials

The average particle size (z-average size) and polydispersity index (PI) were measured by photon correlation spectroscopy (PCS, Nano ZS90 zetasizer, Malvern Instruments Corp, U.K.) at 25 °C under a fixed angle of 90° in disposable polystyrene cuvettes. The measurements were obtained using a He–Ne laser of 633 nm. No multi-scattering phenomenon was observed.

Zeta potential were measured in folded capillary cells using the Nano ZS90 zetasizer. Measurements were performed in distilled water adjusted with a solution of 0.1 mmol/l sodium chloride to a conductivity of 50 μS/cm at 25 °C. The zeta potential values were calculated using the Smoluchowski equation.

Table 1
The composition of the isotretinoin-loaded SLN

IT-SLN	PA5 (wt.%)	SL (wt.%)	Tween 80 (wt.%)	Isotretinoin (wt.%)	Water (wt.%)
A	3.00	6.00	3.00	0.06	87.94
B	3.00	6.00	4.50	0.06	86.44
C	3.00	6.00	6.00	0.06	84.94
D	3.00	4.00	4.50	0.06	88.44
E	3.00	8.00	4.50	0.06	84.44

2.3.2. Transmission electron microscopy (TEM)

TEM was used to characterize the microstructure of IT-SLN. SLN was placed on a carbon-coated copper grid and then a drop of 1% phosphotungstic acid covered on SLN. The superfluous phosphotungstic acid on SLN was wiped off by filter paper. The TEM images were obtained using a Tecnai G2 20 TEM (FEI Corp., German).

2.3.3. Entrapment efficiency (EE)

The isotretinoin-loaded SLN (0.25 g) were separated using Sephadex-G50 column (13.5 cm × 2.0 cm), washing with distilled water at a flow rate of 2.0 ml/min (Zhang et al., 2005). The entrapped and free isotretinoin was respectively collected at continuous volume intervals of 2.0 ml. The collected samples were diluted using a mixed solvent of methanol and chloroform (1:2). EE was calculated according to the following equation:

$$\mathrm{EE} = \frac{\text{the amount of entrapped drug in SLN}}{\text{the amount of entrapped drug in SLN and free drug in dispersion}}$$

2.4. Stability of the isotretinoin-loaded SLN

The chemical and physical stabilities of IT-SLN were evaluated at 2–8 °C for 3 months via clarity, particle size, zeta potential, and HPLC analysis of isotretinoin.

The centrifuge tests were also carried out to assess the physical stability of the isotretinoin-loaded SLN. The isotretinoin-loaded SLN were centrifuged for 30 min at 20 000 rpm in the centrifuge tests.

2.5. HPLC analysis of isotretinoin

Isotretinoin was analyzed by reversed phase HPLC using Agilent 1100 series (Agilent, USA). The HPLC system consists of quaternary pump, an autosampler, a diode array detector (DAD detector) and a workstation. The column was an Inertsil ODS-3 C_{18} column (5 μm, 4.6 mm ID × 15 cm, GL Sciences Inc.). The column temperature was set at 40 °C. The mobile phase was a methanol–water–glacial acetic acid (79:20:1 v/v) mixture with a flow rate of 2.0 ml/min. The detection wavelength was set at 356 nm. The assay was linear in the concentration range of 0.2–20.0 μg/ml. The recovery rate ranged from 98.3% to 100.4%. The R.S.D. value for precision is below 2%. No interference from the formulation or skin tissue was observed. All samples were filtered through an aqueous 0.45 μm pore size filter membrane in order to protect the column.

2.6. In vitro skin permeation studies

The full-thickness abdomen skin from rats was used for all the permeation experiments. After hair was removed with a shaver, excised and examined for integrity using a lamp-inspecting

method, the skin was rinsed with physiological saline. The fat tissues below skin were carefully chopped. The thickness of each skin was similar.

The skins were clamped between the donor and the receptor chamber of vertical diffusion cell with an effective diffusion area of 2.8 cm^2 and a cell volume of 7 ml. The receptor chambers were filled with freshly mixture of physiological saline and 95% ethanol (7:3 v/v). Ethanol was used to solubilize isotretinoin. The diffusion cells were maintained at 37 °C using a re-circulating water bath and the fluid in the receptor chambers was stirred continuously at 300 rpm. The formulations (1.0 g) were gently placed in the donor chambers. At 1–8 h, 0.5 ml of the fluid in the receptor chambers were sampled for HPLC determination and replaced immediately with an equal volume of freshly mixture of physiological saline and 95% ethanol (7:3 v/v). Each preparation was studied three times and the result of each preparation is the average value of three experiments.

The cumulative amounts of isotretinoin permeated through rat skins were plotted as a function of time. The permeation rate of isotretinoin at steady state (J, $\mu g\ cm^{-2}\ h^{-1}$) through skins was calculated from the slope of the linear portion of the cumulative amount permeated per unit area versus time plot.

At the end of the experiments, the skins were removed and rinsed with distilled water. Then, skins were respectively soaked in 1 ml of methanol for 24 h and were subjected to five sonication cycles of 30 min each in a KQ-100 ultrasound bath (Kunshan, China), followed by centrifugal separation. The skin accumulative amount, namely the total amount of isotretinoin extracted from skin at the end of the permeation study (at 8 h) could be obtained from the concentration of isotretinoin in supernatant methanol (Baroli et al., 2000; Chen et al., 2006). The extracted isotretinoin from skins was analyzed by HPLC. It was observed that the sonication had no influence on the stability of isotretinoin. No HPLC peak signal of isotretinoin in the residual extracted skins was observed and showed a high extraction ratio.

2.7. Data analysis

All the skin permeation experiments of each preparation were repeated three times and data were expressed as the mean value ± S.D. The statistical data were analyzed using nonparametric test with a Wilcoxon test. $P < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Preparation of isotretinoin-loaded SLN

In order to disperse isotretinoin homogeneously in the melted lipid, ethanol was used as the solvent. A high speed stirring was employed to obtain a pre-emulsion before homogenization. A hot water bath was used for maintaining the pre-emulsion above the melting point.

In order to select a suitable lipid for isotretinoin-loaded SLN, Compritol 888 ATO, PA5 and glycerin monostearate (GMS) were respectively used as the lipids of SLN. We found that all the lipids could result in translucent dispersion. However, Compritol 888 ATO-based SLN had a large particle size and the GMS-based SLN had a wide size distribution. Both SLN showed poor stability due to large particle size and wide size distribution. The PA5-based SLN showed a suitable size, size distribution and physical stability. Then, PA5 was selected as the optimum lipid for isotretinoin-loaded SLN.

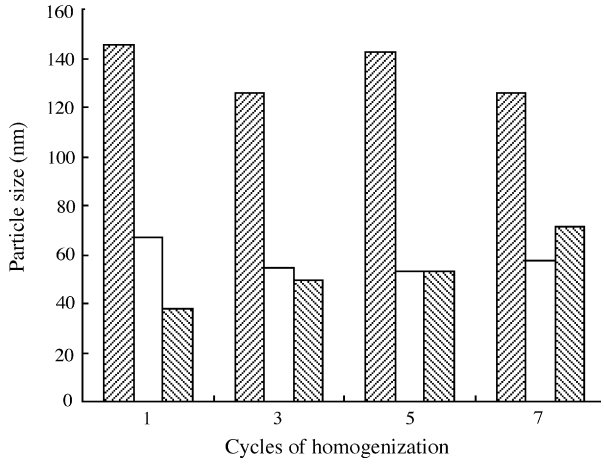

Fig. 1. Particle size of isotretinoin-loaded SLN at different pressure with different cycles of homogenization: (▨) 500 bar, (□) 800 bar, and (▧) 1000 bar.

In addition, the pressure and cycle times of homogenization were also investigated. The particle size of IT-SLN at 500, 800, 1000 bar with 1, 3, 5 and 7 cycles of homogenization are shown in Fig. 1. IT-SLN homogenized at 500 bar showed a relatively large particle size and also an irregular change of particle diameters with the increase of homogenization cycles. So far the reason for this irregular change is unexplained. The pressure of 1000 bar resulted in low particle size, but there is a slight increase of particle size of IT-SLN with the increase of the homogenization cycles. Maybe the high pressure of homogenization might result in the formation of the small particles and then the small particles could aggregate to form a large nanoparticle because of the absence of enough surfactants. IT-SLN homogenized at 800 bar with five cycles showed a narrow distribution and small diameters and then was selected as the optimum pressure for constructing the IT-SLN formulations.

3.2. Characterization of IT-SLN

3.2.1. Particle size

The different IT-SLN formulations (Table 1) were respectively measured by PCS. The particle size and PI were respectively shown in Table 2. The average size of all the formulations

Table 2
The physicochemical properties of IT-SLN

IT-SLN	Particle size	PI (nm)	Zeta potential (mV)	EE (%)
A	46.95 ± 4.23	0.254 ± 0.013	−7.94 ± 1.67	90.49 ± 0.36
B	37.33 ± 3.71	0.179 ± 0.017	−15.97 ± 1.12	95.59 ± 0.30
C	31.27 ± 3.19	0.234 ± 0.021	−14.13 ± 2.25	97.06 ± 0.33
D	42.7 ± 5.50	0.258 ± 0.016	−13.73 ± 1.51	82.62 ± 0.42
E	50.04 ± 4.82	0.275 ± 0.023	−17.85 ± 2.63	99.70 ± 0.27

located between 30 and 60 nm. IT-SLN B had the most narrow size distribution of 0.179 and IT-SLN C has lowest particle size of 31.27 nm, compared with the other formulation. The particle size was decreased with the increase of the concentration of Tween 80. But SL showed an optimum concentration when the concentration of SL in SLN was increased. In addition, no significant difference of the distribution between IT-SLN and drug-free SLN was observed. The incorporation of isotretinoin into drug-free SLN only resulted in a slight change of average size. May be it is due to the extremely low amount of drug.

3.2.2. Zeta potentials

The zeta potentials of IT-SLN are presented in Table 2. The zeta potentials of all the formulations are about −15 mV. The incorporation of isotretinoin into SLN showed no influence on the zeta potentials of nanoparticles. Even though a high zeta potential can provide an electric repulsion, Tween 80 also provide a steric stability for maintaining the stability of SLN (Lim and Kim, 2002).

3.2.3. TEM imaging

The TEM imaging of IT-SLN D is shown in Fig. 2. The particle size of IT-SLN from TEM images accords with that from that from PCS. The imaging showed that IT-SLN D exhibited a spherical shape and had a narrow size distribution.

3.2.4. Entrapment efficiency

The drug EE was measured using Sephadex G50 column and all the IT-SLN formulations had high EE (Table 2). The concentration of surfactants (Tween 80 and SL) showed a significant influence on EE. IT-SLN E with high concentration of SL exhibited the highest EE of 99%. The EE of IT-SLN D is 82.68% and the other IT-SLN formulations are higher than 90%. The low concentration of isotretinoin in SLN and the high compatibility between drug and lipid might resulted in the high EE. The high EE might be beneficial to reduce the skin irritation of drug due to avoid the direct contact between drug and skin surface.

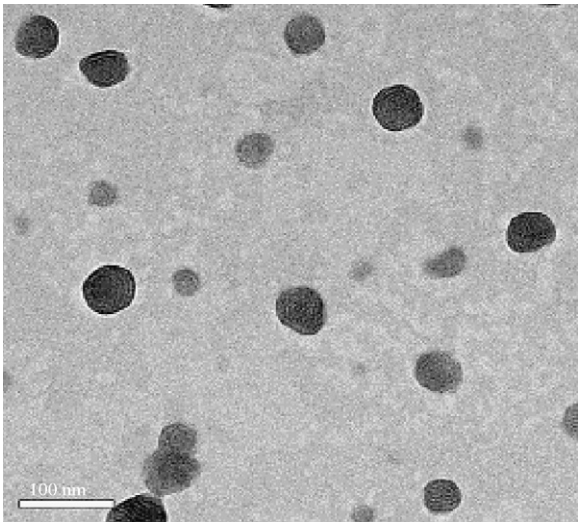

Fig. 2. The TEM imaging of IT-SLN D.

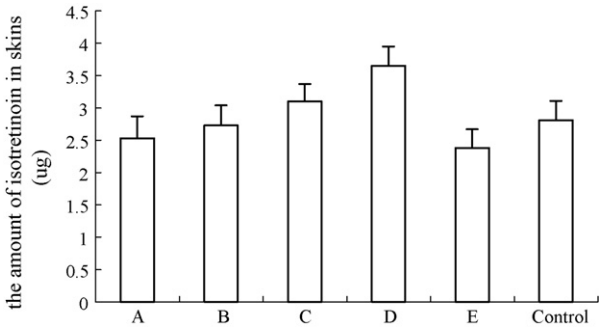

Fig. 3. The accumulative amount of isotretinoin in skins from IT-SLN at the end of the in vitro permeation studies.

3.3. Stability of SLN

IT-SLN showed a good stability during the period of 3 months. No significant change of clarity and phase separation was observed. The centrifuge test also showed that IT-SLN had a good physical stability. The good stability might derive from the slow transition of lipid in SLN, low particles size and the steric effect of Tween 80 (Venkateswarlu and Manjunath, 2004). No degradation of isotretinoin in formulations was also observed.

3.4. Studies of in vitro permeation

In order to assess the skin uptake and penetration of isotretinoin from SLN, the in vitro permeation ability through skins and into skins were performed using Franz diffusion cells (Trotta et al., 2003). The tincture containing 0.06% isotretioin was used as a control to evaluate the skin targeting ability of the IT-SLN formulations. The permeation studies were performed for 8 h according to the clinical application time. It indicated that isotretinoin was not found in receptor chambers from IT-SLN and all the IT-SLN formulations could not penetrate through skin. But the amount of isotretinoin in receptor chamber from the control tincture was increased with increase of time and the control showed a steady permeation rate of $0.76 \pm 0.3\ \mu g\ cm^{-2}\ h^{-1}$. The permeation followed zero order release kinetics. The high permeation rate of isotretinoin from tincture might be due to the significant permeation enhancement effect of ethanol. In addition, during the permeation studies, the loss of ethanol can enhance the concentration of isotretinoin in tincture, also followed by the increase of the thermodynamic activity of isotretinoin (Wissing et al., 2001). These factors might contribute to the penetration through skins from tincture. It is concluded that all the IT-SLN formulations can avoid the systemic uptake of isotretinoin in comparison with the control and SLN present a potential to avoid the systemic adverse side effect. The accumulative amounts of isotretinoin in skins from the IT-SLN A, B, C, D, E and tincture were respectively 2.53, 2.73, 3.10, 3.65, 2.38 and 2.81 μg (Fig. 3). There is no significant difference of accumulative amount of isotretinoin in skin between IT-SLN A, B, C, E and tincture. Even though IT-SLN A, B, C and E failed to increase the uptake of drug in skins significantly when compared with tincture, they still avoided systemic absorption of isotretinoin and showed skin targeting. The IT-SLN D sig-

nificantly increased the accumulative uptake of isotretinoin in skin, compared with the other formulations ($P < 0.01$). IT-SLN D showed 30% and 17% increase of uptake of drug in skins over control and IT-SLN C, respectively, and had an enhanced skin targeting effect. There are some similar results indicating that SLN can increase the uptake of drug in skins (Maia et al., 2000). In our previous work, the skin targeting effect was disclosed by fluorescence microscopy (Chen et al., 2006). SLN with small diameters are advantageous to improve the penetration of nanoparticles into skins and the controlled release of SLN may induce the increase of drug accumulation (Cevc, 2004). In this work, the permeation studies also supported the previous results. However, all the IT-SLN formulations had small diameters. The slight difference between the average particle size of IT-SLN formulations should not be the key factors influencing the uptake of drug in skins. According to Fig. 3, IT-SLN D had the lowest concentration of SL, but had the highest uptake of isotretinoin in skins when compared with IT-SLN B and E. The increase of SL in formulations resulted in the decrease of the accumulative amount of isotretinoin in skins. Additionally, the increase of Tween 80 in IT-SLN A, B and C led to the increase of the uptake of isotretinoin in skins. It is concluded that the concentration of the ingredients of SLN had influence on the uptake of drug in skins. In addition, IT-SLN D had only EE of 82.62%, but showed a high skin targeting. EE might also act as an important factor for the uptake of isotretinoin in skins. Even though the occlusive effect succeeds to elucidate the penetration of drug from into skins, the skin targeting mechanism is unclear and the relative mechanism need further investigation in future (Souto et al., 2004; Williams and Barry, 2004).

4. Conclusions

The various IT-SLN formulations were prepared by hot homogenization method for topical delivery of isotretinoin. The in vitro permeation studies showed all the formulations could avoid the systemic uptake of isotretinoin when compared with the control. IT-SLN D had high accumulative amount of isotretinoin in skins and showed a significant skin targeting effect. The EE and the concentrations of the ingredients of formulations could influence the uptake of drug. The suitable mechanism for skin targeting needs still further studies.

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