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Characterization and Body Distribution of β-Elemene Solid Lipid Nanoparticles (SLN)

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ABSTRACT Solid lipid nanoparticles (SLN) containing β-elemene, a volatile oil used for the treatment of cancer, were prepared by the method combining probe sonication and membrane extrusion. Effects of the formulations and procedures on the characteristics of SLN were investigated. Body distribution of β-elemene SLN in rats after intravenous administration was compared with that of the commercial emulsion. The results showed that dispersing the surfactant in the melted lipid matrix could obtain smaller particles than that dispersing in the water phase. Increasing the ratio of monostearin in the lipid matrix or the concentration of surfactant reduced the mean volume size of the SLN. Optimized formulation was composed of monostearin and precirol ATO 5 at a mass ratio of 3:7, which was quite stable for 8 months at room temperature. In vitro release of β-elemene from the SLN was slow and stable without obvious burst release and was found to follow the Higuich equation. After intravenous administration, the β-elemene levels after 5 min injection of SLN formulation were 1.5, 2.9, and 1.4 times higher than those of β-elemene emulsion in liver, spleen, and kidney, respectively, while the concentrations of β-elemene were decreased 30% in heart and lung. Therefore, the SLN containing β-elemene might be an attractive candidate for the treatment of liver cancer.

KEYWORDS Solid lipid nanoparticles (SLN), Volatile oil, β-elemene, Body distribution

INTRODUCTION

β-elemene, an anticancer compound, is a sesquiterpene extracted from the rhizome of *Curcuma zedoaria (Berg.) Rosc* (Guo et al., 1983). It can inhibit the cancer by inducing the apoptosis of the cancer cell and at the same time enhancing the immunity of the patients (Guo et al., 2003). Injectable emulsion of β-elemene has been developed and found clinical use on the therapy of cancers of lung, brain, and ascites or hydrothorax caused by cancer (Shen et al., 1997). However, it can cause phlebitis in some population after i.v. administration and gives a relatively low bioavailability after oral

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administration, which had severely limited the broad use of it (Wang & Xie, 1997). Efforts should be made to find alternative system.

Solid lipid nanoparticles (SLN) introduced as an alternative colloidal drug carrier to emulsion, liposome, and polymeric nanoparticles in 1991, have attracted increasing attentions in recent years (Wissing et al., 2004). Having a physiological lipid matrix that could melt when heated, SLN could easily achieve the goal of large-scale production, low/non biotoxicity and avoidance of organic solvents. Moreover, the matrix is solid at body temperature, which enables SLN to possess some advantages of polymeric nanoparticles such as increased drug stability, high drug payload, and incorporation of lipophilic drugs. Different kinds of drugs have been incorporated in SLN, including lipophilic and hydrophilic drugs, small molecular and large molecular biological drugs, but SLN containing volatile oil has seldom been reported. Being incorporated in the solid matrix of the SLN, β-elemene might be well protected and realize targeting release different from that of commercial emulsion.

In this study, β-elemene incorporated SLN were prepared by the method of sonication and membrane extrusion. The effects of formulation and procedure parameters on the quality of SLN were examined. The particle size, zeta potential, drug concentration, entrapment efficiency, long-term stability, and in vitro release of the optimized β-elemene SLN were investigated. Body distribution of β-elemene SLN in rats after i.v. injection was compared with that of the commercial emulsion.

MATERIALS AND METHODS

Materials

Monostearin was purchased from Changsha Chemical Reagent Co., Ltd., China. Precirol ATO 5 (Glycerol palmitostearate) was a kind gift from GATTEFOSSE, France and it is a mixture of approximately 8–22% mono-, 40–60% di-, and 25–35% triglycerides of acid (C16,C18), fatty acids other than acid (C16,C18) account for less than 10%. The melting point lies between 50° and 60°C. Lutrol F 68 (poloxamer 188) was donated by BASF, Germany. Acetonitrile was chromatographic grade. β-elemene was from Dalian Medicine Research Institute. All the other chemicals were of analytical reagent grade.

Preparation of β-Elemene Solid Lipid Nanoparticles

Two methods were used for the preparations of SLN; one was adding the surfactant into the lipid matrix (SL), the other was adding the surfactant into the water phase (SW). In the first method (SL), lipid matrix and Lutrol F 68 were melted and mixed at 75°C in a water bath. β-elemene was dispersed quickly in the melted mixture and poured into a bi-layer glass tube with the outer space allowing water to flow at 60°C, then double-distilled water (10 ml) of 60°C was added. The mixture was sonicated by the probe for 10 s and then the energy was switched off for 10 s. The procedure was repeated until the SLN had a total sonication time of 6 min. The dispersion was drawn with a syringe and filtrated through a 0.22 μm microporous membrane during which the temperature was kept at 60°C by circulated water. Subsequently, the filtrate was cooled at room temperature.

In the second method (SW), the surfactant was not mixed with the lipid but dissolved in the double-distilled water. The other procedures were similar to that of the first method. All the SLN were prepared by method of SL unless specially indicated.

Measurement of Particle Size and Zeta Potential

Size of SLN was determined by photon correlation spectroscopy using a Nicomp^{TM} 380ZLS (Particle Sizing Systems, Santa Barbara, CA) equipped with an autocorrelator and a small He–Ne laser (632.8 nm). Each sample was diluted to a suitable concentration with double-distilled water. Dynamic light scattering data were collected in the drop-in cell mode at 23°C at an angle of 90°. The mean diameter was determined by volume mean diameter.

The zeta potential of SLN in suspension was detected by DELSA440SX (BECKMAN Coulter, USA). Samples were diluted with double-distilled water. The conductivity of the diluted sample was measured to choose the detection model. The whole measurement was carried out at 25°C.

Determination of Concentration and Entrapment Efficiency of β-Elemene in SLN

A HPLC assay method was established to determine the concentration of β-elemene in the in vitro and in vivo experiments. The chromatographic system consisted of liquid chromatograph (LC-6A, Shimazu), UV-VIS spectrophotometric detector (SPD-6AV Shimazu), column oven (CTO-6A, Shimazu), and system controller (SCL-6A, Shimazu). N-2000 chromatogram data workstation (Zheda Zhida Intelligentized Informational Engineering Co., Ltd.) was used. The separation was carried out on a Hypersil BDS C_{18} column (5 μm, 4.6 mm × 200 mm) by using acetonitrile-water (88:13) as the mobile phase and the flow rate was set at 1 ml/min^{-1}. The detection wavelength was 210 nm and the column temperature was 30°C. Injection volume was 20 μl. Under these conditions, the linear calibration curve of β-elemene was in the range of 10.6–53.0 μg/ml^{-1} for the in vitro experiment and 0.5–24.8 μg/ml^{-1} for the detection in plasma or tissues.

Aqueous dispersion of SLN (40 μl) was diluted to 10 ml with dichloromethane/ethanol (1:4) to get a clear and uniform solution to determine the concentration of β-elemene in SLN preparations.

After Sephadex G-50 column chromatography of the aqueous dispersion, free β-elemene was separated from those that entrapped in the SLN using distilled water as eluent. Amount of entrapped β-elemene in SLN was detected by HPLC after addition of dichloromethane and ethanol to get a clear solution.

In Vitro Release of β-Elemene from SLN

Dialysis method was used for the in vitro release study. Briefly, 100 μl SLN suspension (5.6 mg/ml) was placed in 10 dialysis tubes (MW cutoff 12,000–14,000 Da) respectively, ligated and immersed in the release media of 36% alcohol (2500 ml) stirred by magnetic force at 25°C. Samples were taken out at fixed time intervals. Aqueous dispersion in the dialysis bag was transferred into a 10 ml volumetric flask, the remains in the bag were washed into the same volumetric flask with a little amount of water and anhydrous alcohol. Then, 2 ml dichloromethane was added into the flask and shook until a clear solution was obtained; anhydrous alcohol was added to the scale. The concentration of β-elemene was assayed by HPLC.

Stability of β-Elemene Loaded SLN

Ampoules of β-elemene SLN were stored at room temperature (18–28°C) under the protection of light for up to 8 months. Samples were characterized at 1 day and 8 months, respectively, as described above concerning the mean volume diameter, drug concentration, zeta potential, and entrapment efficiency.

Pharmacokinetic and Body Distribution Studies

Animal experiment was performed according to the Guidelines for Animal Experimentation of Shenyang Pharmaceutical University. The Wistar rats (220 ± 20 g, male) were purchased from the Laboratory Animal Center of Shenyang Pharmaceutical University (Shenyang, China). The rats were kept under standard conditions, with free access to water and food. The β-elemene emulsion and aqueous dispersion of SLN after iso-osmotic adjustment by glucose were administered intravenously via the dorsal tail vein. Five rats were treated with a single dose equivalent to 44.2 mg/kg^{-1} (1 ml/100 g).

In the pharmacokinetic studies, blood samples (0.2 ml) were taken from the retro-orbital plexus of the rats at the predetermined time (5, 10, 20, 30, 40, 50, 60 min) after injection. Plasma was immediately separated by centrifugation and stored at −20°C before analysis. In the tissue distribution studies, the rats were sacrificed after collection of the blood and tissues (heart, kidney, liver, lung, spleen, and brain) were excised, washed with physiological solution and frozen to −20°C until use. Accurately weighted tissue specimen was placed in a homogenizing tube with double volume of double distilled water and homogenized in an ice-bath. The homogenate or the plasma was diluted with double volume of methanol and vortex mixed for 5 min. After centrifugation at 10,000 × g for 5 min, 20 μl of the supernatant was analyzed by HPLC immediately.

TABLE 1 Effect of Surfactant Adding Methods on the Mean Particle Size, Zeta Potential, and Entrapment Efficiency of SLN

Lipid composition (W/W)	Size (nm)	Zeta potential (mV)	Entrapment efficiency (%)
100%A[a]	**106.5±3.9**[c]	**−32.5±3.7**	**99.3±1.6**
100%A[b]	149.7±3.3	−29.8±3.8	97.2±2.0
(70%A+30%D)[a]	**46.7±2.0**[c]	**−34.9±3.1**	**99.8±2.1**
(70%A+30%D)[b]	51.5±2.2	−32.7±3.2	96.9±2.4
100%D[a]	**26.5±1.6**[c]	**−33.7±3.4**	**99.9±1.6**
100%D[b]	32.8±1.3	−35.3±3.5	98.2±1.5

A means Precirol ATO 5. D means monostearin. SLN were prepared with 2.5% (w/v) Lutrol F 68, 5% (w/v) lipid in the presence of 5.6 mg/ml β-elemene. Results represent the mean±S.D. of three samples.

[a]Adding the surfactant into the lipid phase during preparation.

[b]Adding the surfactant into the water phase during preparation.

[c]$P<0.05$ compared with that prepared by method of SW.

The pharmacokinetic parameters, AUC_{0-t} (area under the plasma concentration-time curve from time zero to the time of last measurable concentration), $AUC_{0-\infty}$ (area under the plasma concentration-time curve from time zero to infinity), $t_{1/2}$ (plasma half-life), Cl_{tot} (total body clearance), V_d (apparent volume of distribution), and MRT (mean residence time) were computed by a non-compartmental model applying the program Topfit, version 2.0 (Thomae GmbH, Germany).

Statistical Analysis

Statistical analysis was performed using Student's t-test, and the differences were considered significant at a confidence level of 95% ($P<0.05$).

RESULTS AND DISCUSSION

β-elemene SLN was prepared by the method combining the probe sonication and membrane extrusion techniques. Short time of sonication was used to realize preliminary dispersion of the lipid matrix followed by membrane extrusion at high temperature to further homogenize the particles and exclude the microparticles.

Probe sonication is a very effective method to reduce the size of large particles in a small volume. It has been widely used to produce small vesicles such as liposome, polymeric nanoparticles, and SLN due to the widespread and maneuverable equipment (Mei et al., 2003; Park et al., 2004; Pellequer et al., 2004). However, there still exist some drawbacks inhibiting its use. One is that it could not efficiently reduce the number of large particles, and the other is prolonged sonication would aggregate the risk of lipid and drug degradation and contamination with titanium from probe. The membrane extrusion technique could be adopted after sonication to reduce the sonication time and improve the quality of the SLN.

Additionally, this method has special advantages for the production of drugs with high volatility. β-elemene is a volatile oil with a boiling point of 117–124°C and a poor chemical stability. Long time exposure of drug to light, high temperature should be avoided to decrease the drug degradation or volatilization. In the production of β-elemene SLN, low temperature and short time of sonication and heating was adopted. In addition, the filtrate could be sterilized after filtration through 0.22 μm pore of membrane.

Effect of Surfactant Adding Method

Surfactant has shown a great impact on the quality of the SLN (Lim & Kim, 2002). Not only the amount but also the distribution of the surfactant in the SLN dispersion will affect the characteristics of SLN. In the same formulation, there exists at least two choices of adding the surfactant. One is adding it into the water phase during preparation which was commonly adopted in the hot homogenization method (Mehnert & Mader, 2001), and the other is mixing the surfactant with the lipid. However, no comparison of them has been reported. We investigated the effect of these two methods on the size, zeta potential, and entrapment efficiency of the SLN.

Three formulations were prepared by the two methods, respectively, and their properties are listed in Table 1. The mean volume diameter of SLN prepared by SL were significantly smaller than that

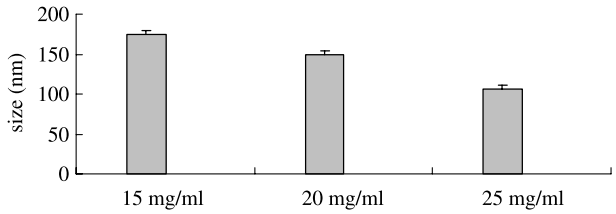

FIGURE 1 **Effect of Surfactant Concentration on the Particle Size of the β-Elemene SLN. SLN Containing 5.6 mg/ml β-Elemene were Prepared with 5% (w/v) Precirol ATO 5 and Varying the Amount of Lutrol F 68. Results Represent the mean ± S.D. of Three Samples.**

prepared by SW of the same formulation, suggesting a more efficient dispersing effect of SLN prepared by method of SL. This result was mainly attributed to the comprehensive velocity of the particle partition and the coverage of the new lipid surfaces by the surfactant during the preparation, which will determine the final size of the SLN. In the preparation of SLN by method of SL, large amount of surfactant in the lipid phase will facilitate the particle partition compared with the method of SW and have the prerequisite of obtaining small particles. However, the newly formed surface of the particles must be protected by the surfactant at a certain speed in order to compete with the agglomeration of uncovered lipid surface. The low concentration of surfactant in the water phase may not favor the process of physical absorbance on the new surface, but the surfactant homogenously dispersed in the matrix could quickly protect the newly formed surface nearby and the more hydrophilic part of the surfactant will move to the surface of the particles to have a more effective protection. In the SLN prepared by method of SW, the high amount of surfactant in the water phase could quickly cover the new surfaces, but the efficiency of disruption was smaller than that of matrix containing surfactant and led to larger particles.

The entrapment efficiency of SLN was also affected by the method. As a lipophilic drug, β-elemene was well entrapped in all the preparations with higher entrapment efficiency. While compared with the SLN prepared by method SW, the SLN prepared by method of SL showed a tendency of more efficient incorporation of the drug. This is probably due to the different concentration of surfactant in the water phase. In the method of SW, surfactant was added directly into the hot water to protect the particles from aggregation mainly by physical absorbance on the surface during production and storage. However, the large amount of superfluous surfactant in aqueous phase will inevitably cause the partitioning effect of the drug between the solid lipid phase and the aqueous phase, which will reduce the entrapment efficiency or form a drug-enriched shell (Muller et al., 2000). The concentration of surfactant in the aqueous phase prepared by method of SL might be low, which means that most of the drug could only distribute in the lipid phase or be absorbed on the surface of the SLN. In addition, the coexistence of some colloidal species caused by the redundant surfactant in water phase might be reduced (Mehnert & Mader, 2001).

Zeta potential was not obviously affected by the methods. The measurement of zeta potential allows prediction on the stability of colloidal aqueous dispersion (Komatsu et al., 1995). Particle aggregation is less likely to occur for charged particles with absolute values of zeta potential larger than 30 mV due to electric expulsion (Levy et al., 1994). Although all of these freshly prepared formulations had high zeta potential, the physical stability could only be

TABLE 2 **Effect of Percentage of Monostearin in the Lipid Matrix on the Mean Particle Size and Long Stability**

	Mean volume size (nm)	
Percentage of monostearin in the lipid	1 day	8 months
0%	106.5 ± 3.9[a]	357.1 ± 20.9
10%	86.4 ± 2.6[a]	114.4 ± 10.2
30%	46.7 ± 2.0	48.9 ± 2.6
50%	33.5 ± 1.5	36.2 ± 2.8
100%	26.5 ± 1.6	——[b]

SLN containing 5.6 mg/ml β-elemene were prepared with 2.5% (w/v) Lutrol F 68 and 5% (w/v) lipid matrix composed of monostearin and Precirol ATO 5. Results represent the mean ± S.D. of three samples.

[a] $P < 0.05$ compared with that after 8 months.

[b] Not detected because of aggregation.

TABLE 3 Characteristics of β-Elemene SLN

Storage time	Volume mean size (nm)	Zeta potential (mV)	Drug concentration (mg/ml)	Entrapment efficiency (%)
1 day	46.7±2.0	−34.9±3.1	5.6±0.1	99.8±2.1
8 months	48.9±2.6	−30.7±4.5	5.6±0.2	99.7±2.5

Each value represents the mean±S.D. of three samples.

ensured after a long-term investigation. There exist many factors that might influence the stability of the SLN, such as the phase transition of perfect lattice during storage will change the surface property of the SLN (Mehner et al., 1997).

Effect of Surfactant Concentration and Ratio of Monostearin in the Lipid Matrix on the Size and the Stability

In general, dispersion with a high concentration of surfactant can reduce the surface tension and facilitate the particle partition during homogenization (Schwarz et al., 1994). This is true for the preparation of β-elemene SLN. Increasing the concentration of surfactant from 15 mg/ml to 25 mg/ml, the mean volume diameter reduced significantly (Fig. 1).

Despite a high drug loading ability, the physical stability of SLN produced by monostearin was poor (Jenning & Gohla, 2000; Zimmermann et al., 2000). Precirol ATO 5 can only maintain the dispersing state for a long time with no or low loading of β-elemene. Mixture of these two components at optimal ratio might realize an improved physical stability with a high drug loading. SLN composed of different ratio of Precirol ATO 5 and monostearin were prepared and investigated.

Table 2 showed the influence of monostearin content in the lipid matrix on the particle size of the SLN. Replacing 10, 30, 50, and 100% of the Precirol ATO 5 with the monostearin could reduce the diameters gradually. This may be caused by the different surfactant property of the lipids. Compared with Precirol ATO 5, monostearin owns more hydrophilic groups and possesses higher self-emusification ability which could help to improve the dispersing ability of the formulation.

The physical stability of SLN was also affected by the ratio of monostearin. After storage of eight months, the particle size of SLN with 100% Precirol ATO 5 was about three times larger than that of the original with certain amount of microparticles. Replacing 10% of the Precirol ATO 5 with monostearin reduced the aggregation but the particle size significantly increased. Increasing the percentage of monostearin to 50%, the particle size increase was not significant and few large particles were detected by PCS. SLN composed of 100% monostearin aggregated into large particles and could not maintain the uniform dispersion state. The instability of β-elemene SLN composed of Precirol ATO 5 might be similar to that of Compritol 888 ATO (a mixture of mono-, di-, and triglycerides of behenic acid) (Jores et al., 2004). Some droplets of β-elemene might absorb on the surface of the nanoparticles, reducing the area protected by the surfactant and make the aggregation more easily. In addition, the presence of liquid drug in the lipid phase could promote the transition of the crystals into a stable form because unstable crystals may redissolve and crystallize in a stable modification (Yoshino et al., 1983). Among these formulations, the most stable one is composed of 30% monostearin and 70% Precirol ATO 5, which has no significant change in the size and appearance. Increasing the ratio of monoglyceride for 30% might offer more room to accommodate the β-elemene in the lipid matrix and at the same time own the property to maintain the dispersing state.

Characteristics of Optimized β-Elemene SLN Formulation

Long Term Stability

From the above-mentioned studies, optimized formulation of β-elemene SLN was selected. Precirol ATO 5 (350 mg) was used as the main lipid matrix and monosterin (150 mg) as an adjuvant to improve the stability of the carrier. Lutrol F68 (250 mg) was selected as the surfactant, which was mixed together with the melted lipids and drugs during the preparation.

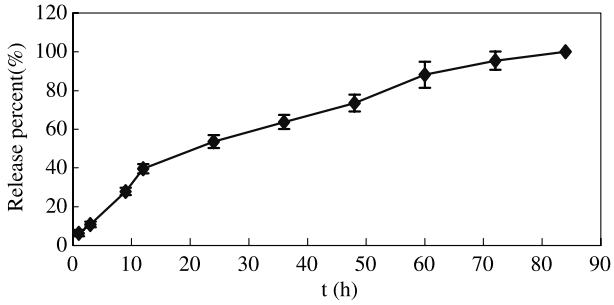

FIGURE 2 **In Vitro Release of β-Elemene from SLN Containing 5.6 mg/ml β-elemene. Error Bars Indicate mean ± S.D. (n=3).**

The size, zeta potential, concentration, and entrapment efficiency of β-elemene in the SLN dispersion were measured at the first day and 8 months after production, respectively (Table 3). In the freshly prepared samples, the particles were distributed in the nanometer range with a high negative zeta potential. About 99% of the particles had volume mean diameters smaller than 132.9 nm, which showed that the β-elemene SLN produced by the method maintained most of their size below the pore of the membrane. The concentration of β-elemene in the SLN dispersion was higher than that of the commercial product (44.2 mg/ml) and the drug was well incorporated. Compared with the fresh samples, there was no significant change in the mean volume diameter after 8 months storage and no particles with diameters larger than 1 μm detected. The concentration and the entrapment efficiency of β-elemene in the SLN were also stable after 8 months storage, indicating that the drug was protected well in the lipid matrix.

In Vitro Release of β-Elemene from SLN

The burst release of SLN was commonly reported because SLN could not efficiently avoid the drug diffusing into the water phase or forming drug-enriched shell especially those adding the surfactant directly into the water phase (Muhlen & Mehnert, 1998). Solubility of the drug in the lipid phase and the water phase at different temperatures has great effect on drug distribution. The less the surfactant in the water phase, the less the lipophilic drug distribute into the water phase. In this section, we investigated the in vitro release of the β-elemene from SLN.

Thirty-six percent alcohol solution was chosen as the dialysis medium for in vitro release of β-elemene from SLN. The solubility of β-elemene in 36% alcohol is 12.4 μg/ml, which could fulfill the sink condition without obviously changing the physical state of the SLN. It should be indicated here that when SLN was added into an alcohol solution with a concentration higher than 45%, particle aggregation or dissolving occurred. Percentage of release was determined by measuring the remaining drug in the dialysis bag because of the instability of the drug in the release medium. Figure 2 showed the release percentage of β-elemene from SLN at different time points. The cumulate release percentage of β-elemene from the SLN within 1 h and 24 h was 6.3% and 53.6%, respectively, and the drug could be released completely within 84 h, indicating that burst release was not obvious in our study. The obtained release data were fitted into First order, Higuchi and Weibull equations, respectively, and the results showed that β-elemene release from SLN follows the Higuchi equation better than First-order and Weibull equation (Table 4).

Drug release profile can reflect the distribution of the drug in the matrix. Drug-enriched shell mode or those not completely incorporated in the lipid matrix will have a burst release. Drug-enriched core model may have a first slow but afterwards a little fast and steady release. The release profile of β-elemene from SLN was slow and steady during the experimental time, which suggested that β-elemene might be

TABLE 4 **Nonlinear Fits of β-Elemene Release from SLN**

Equation type	Equation	R^2
First order, $\ln(Q_\infty - Q)$ vs. t	$y = -0.0378x + 4.6874$	0.9372
Weibull, $\ln\ln\left\{1\Big/\left(1-\frac{Q}{Q_\infty}\right)\right\}$ vs. $\ln t$	$y = 0.8714x - 2.9462$	0.9774
Higuchi, Q vs. $\sqrt{t}$	$y = 11.832x - 6.1458$	0.9941

Q_∞ = maximum cumulate release percentage (%).
Q = cumulate release percentage after t(%).
t = release time (h).

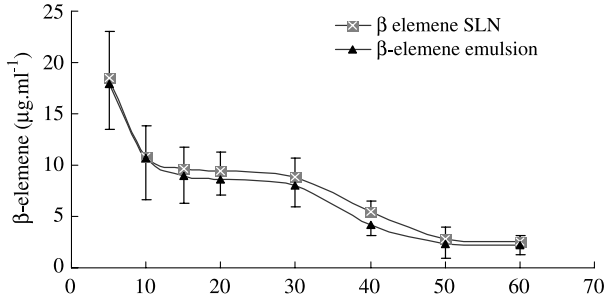

FIGURE 3 **Plasma Concentration-time Profiles of β-Elemene After i.v. Administration of β-Elemene SLN and β-Elemene Emulsion (n=5). Error Bars Indicate mean±S.D. (n=5).**

homogeneously dispersed in the lipid matrix. This result was mainly attributed to the high compatibility of the drug and the selective lipid matrix. Even at a ratio of 1:2 (drug:lipid matrix, W/W), β-elemene could also be incorporated well in the lipid matrix (data not shown). In addition, adding the surfactant into the lipid matrix could reduce the surfactant concentration in the water phase, which inhibited the formation of burst release model.

BODY DISTRIBUTION OF β-ELEMENE SLN AND COMMERCIAL EMULSION IN RATS

The time courses of plasma concentration of β-elemene following i.v. administration of commercial emulsion and SLN in rats are shown in Fig. 3, and the calculated pharmacokinetic parameters are listed in Table 5. The plasma levels of β-elemene after administration decreased rapidly. There was no significant difference between the parameters.

The mean concentrations of β-elemene in selected organs after i.v. administration of emulsion and SLN at different times are shown in Fig. 4. It was clear that β-elemene was distributed into the tested organs rapidly, which explained the fast decreased plasma level of β-elemene. The major organs of accumulation for both preparations are the heart, liver, and kidney, which own a rich blood supply or an abundance of macrophages. The tissue distribution of β-elemene in SLN was different from that of the commercial β-elemene emulsion. The β-elemene levels after 5 min injection of SLN formulation were 1.5, 2.9, and 1.4 times higher than those of β-elemene emulsion in liver, spleen, and kidney, respectively, while lower concentrations of about 70% β-elemene were observed in heart and lung at 5 min after dosing with β-elemene SLN than with β-elemene emulsion. No significant difference in the brain distribution was found.

This result might be attributed to the property of the carriers and the drug. The rapid elimination and wide distribution of the β-elemene emulsion after intravenous injection was in accordance with the report in the literature (Wang & Su, 2000). Colloidal drug delivery systems without specific modification usually show a strong tendency to accumulate rapidly in the phagocytic cells of the reticuloendothelial system (RES) (Barratt, 2000). Similar phenomenon was found in camptothecin SLN (Yang et al., 1999). Compared with the commercial emulsion, SLN showed a higher uptake by RES tissues such as liver and spleen, which indicated that this formulation may be an attractive candidate for treatment of liver cancer. Many SLN preparations have been found to be able to achieve a blood brain barrier (BBB) penetration of drugs (Fundaro et al., 2000; Wang et al., 2002; Yang et al., 1999). β-elemene in both of the preparations could be distributed into the brain and there was no significant difference due to the similar lipophilicity of the carriers. In particular, lower accumulation of β-elemene in the heart for the SLN is an advantage for the treatment of cancers although there is no report on

TABLE 5 **Pharmacokinetic Parameters (Mean±SD) of β-Elemene Following i.v. Administration of β-Elemene SLN and β-Elemene Emulsion in Rats (n=5)**

Parameters	β-elemene SLN	β-elemene emulsion
AUC_{0-t} (μg/min/ml^{-1})	494.6±80.5	456.6±85.6
$AUC_{0-\infty}$ (μg/min/ml^{-1})	551.3±90.1	505.4±106.4
$t_{1/2}$ (min)	15.6±1.2	15.4±1.6
CL_{tot} (ml/min^{-1})	80.0±13.2	87.3±18.3
V_d (L)	1.8±0.3	1.9±0.5
MRT (min)	26.8±1.0	25.6±1.8

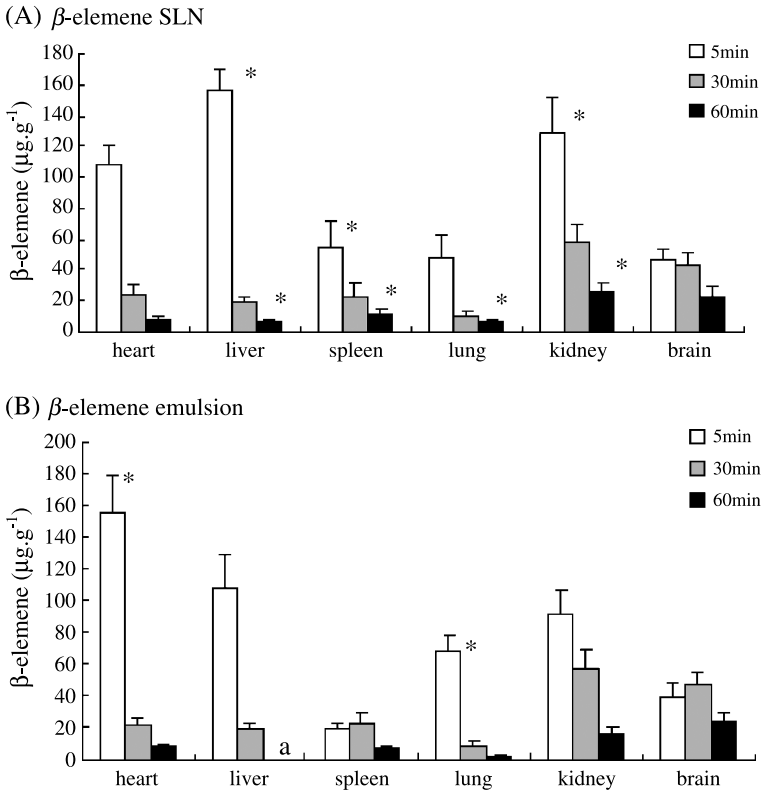

FIGURE 4 **The distribution of β-elemene in rat organs at different time points after i.v. administration of β-elemene SLN (A) and β-Elemene emulsion (B) (n=5). Error bars indicate mean ± S.D. (n=5).**

the cardiotoxicity of β-elemene emulsion. Study of doxorubicin SLN has confirmed that SLN could reduce the accumulation of drug in the heart (Fundaro et al., 2000).

CONCLUSION

β-elemene SLN was prepared by the method combining the techniques of probe sonication and membrane extrusion. It was better to mix the surfactant with the lipid phase during preparation. Using a mixture of monostearin and Precirol ATO 5 at an optimal ratio of 3:7 (W/W) as the lipid matrix, SLN could realize the stability for at least 8 months. The in vitro release was slow and stable without obvious burst release. Following i.v. administration, β-elemene loaded SLN resulted in significant changes in the tissue distribution compared with β-elemene emulsion. The β-elemene levels after 5 min injection of SLN formulation were 1.5, 2.9, and 1.4 times higher than those of β-elemene emulsion in liver, spleen, and kidney, respectively, while lower concentrations of β-elemene were observed in heart and lung at 5 min after dosing with β-elemene SLN than with β-elemene emulsion. Therefore, the SLN containing β-elemene might lead to a reduced toxicity and an increased efficiency of liver cancer therapy.

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