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Preparation, characterization and *in vivo* distribution of solid lipid nanoparticles loaded with syringopicroside

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A solvent emulsification evaporation method was employed to prepare solid lipid nanoparticles (SLN) loaded with syringopicroside. The conventional broad-spectrum antibacterial and antiviral drug syringopicroside was incorporated into SLN to improve drug targeting. The SYR-SLNs were spherical and uniform in transmission electron microscopy (TEM). The mean particle size and potential were 180.31 ± 10 nm, and -41.9 ± 10.3 mV, respectively. Also, a sephadex column chromatography was adopted to investigate the encapsulation efficiency (EE %) of the SLN. This method is based on the principle of molecular sieve effect, and the EE% of the optimal formulation was 42.35 %. Drug-loading capacity was 5.33 %. The *in vitro* release profile revealed that syringopicroside was released from SLN efficiently and completely in normal saline (NS) compared with other release media. A HPLC method was established for *in vivo* assay of syringopicroside. A tissue distribution study was conducted in rats after iv administration of 15 mg/kg SYR-SLN and syringopicroside NS, and it was found that SYR-SLN has improved delivery to the liver compared with any other organizations. These results indicated that solvent emulsification evaporation is a simple, easy, available and effective method for preparing SYR-SLN.

1. Introduction

During the last decade, solid lipid nanoparticles (SLN) have been developed as a promising carrier system for controlled drug delivery. Compared with ordinary particulate carriers, SLN have many advantages, such as good biocompatibility due to the use of physiological lipids, high bioavailability and efficient targeting (Mehnert and Mader 2001). They are also suitable for large-scale production by high pressure homogenization. Therefore, research into SLN for targeted drug delivery systems is currently attracting increasing interest.

Syringopicroside is the monomer ingredient extracted from the leaves of *Syringa oblata* L. *Syringa obalata* L. is a perennial shrub growing in the northeast of China. The leaves have a bitter taste and the properties of broad-spectrum antibiotic, antiviral and immunologic enhancement. Recently, the Chinese system of medicine recommends it as a remedy for bacillary dysentery, inflammatory diarrhea, inflammatory mammary gland, tonsillitis, infection of the upper respiratory tract etc. Nevertheless, clinical observation found that syringopicroside has a larger oral dose, rapid elimination *in vivo* after intravenous injection and low bioavailability. So this thesis was to study the lyophilized powder of syringopicroside loaded solid lipid nanoparticles (SLN) for increasing the role of the liver targeting and enhancing therapeutic efficacy.

In our study, we developed a solvent emulsification evaporation method for the preparation of syringopicroside -SLN (SYR-SLN). With this method, the nanoparticles are obtained by two steps: the formation of an O/W emulsion (aqueous phase including Pluronic F68, combined with a oil phase containing glycerol monostearate (GMS) and yolk lecithin (YL), followed by dispersion of the initial emulsion in cold water including Pluronic F68 and solidification process to obtain a fine particle size. The properties of SYR-SLN, including morphology, particle size, zeta potential, entrapment efficiency (EE%) and *in vitro* release behavior were studied in detail. In addition, the tissue distribution of SYR-SLN and a control formulation in rats were investigated *in vivo* characteristics.

Table 1: Effect of different proportions of the lecithin and glycerol monostearate (n = 3)

YL:GMS	1:1	2:1	3:1	4:1
EE(%)	20.82 ± 0.24	27.88 ± 0.33	37.85 ± 0.30	30.86 ± 0.26

2. Investigations, results and discussion

SYR-SLN was prepared using a solvent emulsification evaporation method. Before preparation, YL was dispersed in distilled water (5% w/v), This method was used in order to enhance the emulsification and aqueous-dispersibility of YL, as previously reported (Giri et al. 2005).

According to our preliminary experiments, through single factor study, we found that the ratio of GMS:YL, organic: aqueous phase, F68% and dosage had significant effects on the appearance and stability of SLN. The statistical data are given in Tables 1, 2, 3 and 4. The influence of GMS on drug loading

Table 2: Effect of different proportions of organic and aqueous phase (n = 3)

O:A	1:2	1:3	1:4	1:5
EE(%)	38.58 ± 0.36	27.2 ± 0.29	32.14 ± 0.32	30.16 ± 0.40

Table 3: Effect of F68 concentration on different dosage (n = 3)

F68(%)	0.2	0.4	0.8	1.6
EE(%)	30.47 ± 0.27	36.44 ± 0.53	31.33 ± 0.42	27.70 ± 0.34

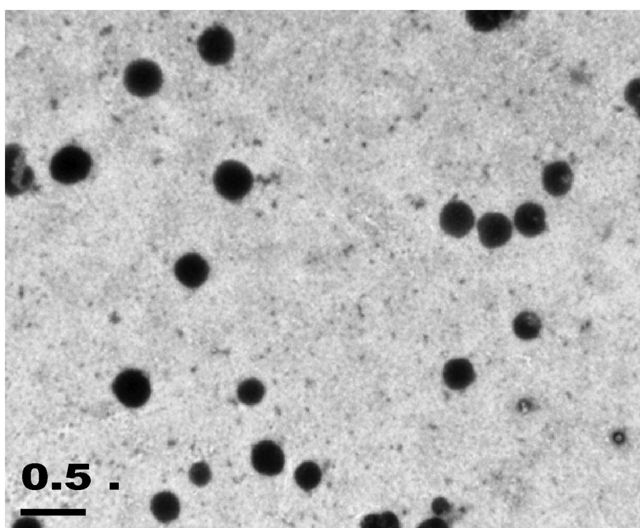


Fig. 1: Photo micrograph of SYR-SLN by TEM (×16500)

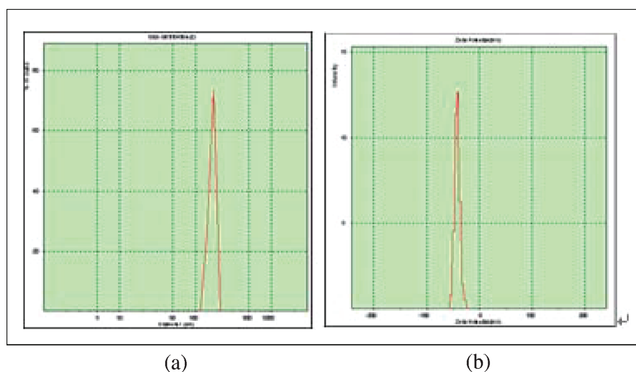
capacity was also marked, the optimal concentration of GMS being 80 mg, while temperature and stirring rate play a relative minor role as far as the properties of the formulation are concerned.

At last, through orthogonal experimental design, an optimum recipes of syringopicroside-SLN was found, that is, lecithin/glycerol monostearate 3:1, organic phase/water phase 1:2, F68% 0.4%, syringopicroside 10 mg. The statistical data are given in Tables 5, 6 and 7.

TEM photography showed that the SYR-SLN particles had subsphaeroidal and uniform shapes (see Fig. 1). The morphology of the SYR-SLN indicated that SYR-SLNs were successfully obtained by the above method.

The average diameter of SYR-SLN obtained was 180 ± 15 nm (n = 3), and the particle size distribution is shown in Fig. 2(a). The SLN used for drug loading exhibited a small particle size and a narrow size distribution. Therefore, stable and highly dispersible nanometric dispersions could be prepared using this method. Fig. 3

The stability of colloidal dispersions is strongly influenced by zeta potential. In general, highly charged particles are less likely to aggregate or flocculate due to electrical repulsion (Müller et al. 2000), and therefore storage stability can usually be increased by an increase in zeta potential. The mean zeta potential of SYR-SLN was –41.9 ± 10.3 mV (n = 3), with a zeta potential distribution as shown in Fig. 2 b.



(a) (b)

Fig. 2: (a) Particle size distribution (b) Zeta potential.

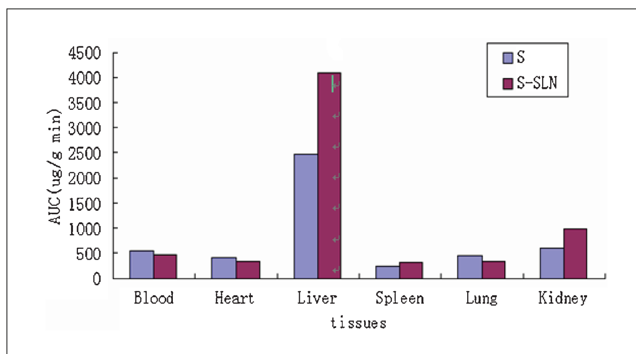


Fig. 3: Area under curve in different tissues after i.v. SS and S-SLN in 1.5 h.

Table 5: Factors and levels

	A YL:GMS	B O:A	C F68	D dosage (mg)
1	1:1	1:2	0.2%	8
2	2:1	1:4	0.4%	10
3	3:1	1:6	0.8%	12

The *in vitro* release profiles of syringopicroside from SYR-SLN were measured in three dissolution media. In all cases, loaded syringopicroside was released within 36 h and no conspicuous burst release was observed in any medium. The release rates in the two PBS were similar with a slight increase at higher pH. It is obvious that the release of syringopicroside from SLN in normal saline was faster than in PBS. Also, the release of SYR-SLN in NS reached 64%, which was much higher than in PBS. These results could be attributed to the high stability and solubility of syringopicroside in NS. As stated above, syringopicroside is easily hydrolyzed in water because of the low concentration of chlorine ions and so is relatively stable in NS. Therefore, the free drug could permeate into NS solution at random, and drug loaded in SLN could be easily released into the receiving phase

Table 4: Effect of syringopicroside loading on different dosage (n = 3)

Dosage(mg)	4	6	8	10	12	14
EE(%)	30.04 ± 0.33	31.67 ± 0.57	33.85 ± 0.68	38.96 ± 0.39	36.32 ± 0.46	29.83 ± 0.92

Table 6: Results of the orthogonal design about SYR-SLN

	A	B	C	D	EE (%)
1	1:1	1:2	0.2%	8	26.51
2	1:1	1:4	0.4%	10	24.70
3	1:1	1:6	0.8%	12	23.50
4	2:1	1:2	0.4%	12	34.56
5	2:1	1:4	0.8%	8	28.25
6	2:1	1:6	0.2%	10	33.88
7	3:1	1:2	0.8%	10	39.19
8	3:1	1:4	0.2%	12	36.67
9	3:1	1:6	0.4%	8	38.90
Ij	24.903	33.420	32.353	31.220	
IIj	32.230	29.873	32.720	32.590	
IIIj	38.253	32.093	30.313	31.577	
Rj	13.350	3.547	2.407	1.370	

Table 7: Results of analysis of variance

Factor	SS	df	S^2	F	P
YL:GMS	268.183	2	88.480	19.000	*
O:A	19.267	2	6.357	19.000	
F68%	10.088	2	3.328	19.000	
dosage	3.031	2	1.000	19.000	
error	3.03	2			

Table 8: Concentration of syringopicroside NS and SYR-SLN in rat after i.v administration (n = 6)

Tissues	C (μg/g) (SLN/NS) 10 min	30 min	50 min	70 min	90 min
Plasma	9.32/20.32	6.73/7.89	4.12/3.43	2.90/1.41	1.10/0.71
Heart	6.72/14.72	4.64/5.73	3.37/2.18	1.12/0.92	0.71/0.63
Liver	62.42/35.49	37.31/14.13	16.92/9.31	8.36/5.42	3.71/1.60
Spleen	6.73/6.73	4.16/3.21	2.71/1.42	1.81/0.62	0.92/0.23
Lung	7.43/15.43	4.23/6.72	3.41/3.14	1.21/1.72	0.87/0.96
Kidney	20.82/40.17	13.41/21.21	9.34/13.71	6.42/8.12	2.43/2.43

through the membrane, due to its high solubility in the release media.

Table 8 shows the concentration of SYR-SLN and syringopicroside NS in plasma, heart, liver, spleen, kidney and lung. Figure 6 shows the area under curve in different tissues after i.v. SYR-SLN and syringopicroside NS. It can be clearly seen that in rats SYR-SLN was mostly concentrated in the liver, while the concentrations of syringopicroside in lung and spleen were much lower than in liver and plasma, with, it should stressed, the lowest in the spleen. Furthermore, compared with syringopicroside NS, the solid lipid matrix plays an important role in protecting the drug from degradation in plasma and organ cells. It is obvious that SYR-SLN showed targeted delivery to the liver.

3. Experimental

3.1. Materials

Syringopicroside was prepared from Heilongjiang University of Chinese Medicine. Glyceryl monostearate (GMS) was obtained from Hunan Erkang Pharmaceuticals Limited Co.(Liuyang, China). Yolk lecithin (YL, for injection) was supplied by Aikang Pharmaceutical Industry Co.Ltd (Shanghai, China). Pluronic F_{68} was kindly donated by BASF(Shanghai, China) .SephadexG-50 was obtained from Pharmacia Co. Ltd (Stockholm, Swiden. Other reagents were of analytical reagent grade.

3.2. Preparation of SYR-SLN

GMS (80 mg) was dissolved in absolute alcohol (5 mL) and heated to 65 °C in a water bath, then YL (240 mg) and syringopicroside (10 mg) were added to the above solvent as the oil phase. The aqueous phase consisted of F_{68} (0.4%) 10 ml which was also heated to 65 °C. The oil phase was poured into the aqueous phase under magnetic stirring (600 rpm) at 65 °C for 60 min. During this process, a nano-emulsion was formed and the absolute alcohol was completely evaporated. The volume was reduced to 5 ml. The nano-emulsion was then dispersed in 0–2 °C water (the ratio of suspension to water 1:1, v/v) under mechanical stirring for 1.5 h to obtain the resultant SYR-SLN suspensions.

3.3. Transmission electron microscopy (TEM)

The morphology of SYR-SLN was examined by TEM (JEM-1200EX, JEOL). The samples were appropriately diluted with distilled water and stained with 2% (w/v) phosphotungstic acid for observation.

3.4. Particle size and zeta potential measurement

The particle size of SLNs in the suspension was determined by dynamic light scattering (DLS) using a laser particle size (Mastersizer S, Malvern, UK). The particle size data were evaluated as the volume distribution. The zeta potential was measured by a zeta potential analyzer (Zatasizer-3000Hsa, Malvern, UK) at 37 °C. Each sample was diluted with distilled water until the appropriate concentration of particles was achieved, and each sample was measured in triplicate.

3.5. Encapsulation efficiency (EE%) determination

The encapsulation efficiency (EE%) of SYR-SLN was determined by a Sephadex G-50 method. In order to assess the feasibility of this method, some experiments were carried out: Add 0.4 ml SYR-SLN suspension to the top of the Sepheadex G-50 column. Then distilled water was added to elute 1d/3 s. At the same time, nanoparticles and free drug were separated. Collected the part of free drug and metered volume to 25 ml, then filtered through a 0.45 μm.It is measured by HPLC. Eq. (1) was used to calculate EE%. Eq. (2) was used to calculate DL%.

$$EE\% = W - W_1/W \times 100\% \quad (1)$$

$$DL\% = W - W_1/W_p \times 100\% \quad (2)$$

EE:Entrapment efficiency, DL: Drug loadings; W: Totle drug of the suspension; W_1:Free drug; Wp: Totle carrier material

3.6. In vitro release study

Drug release from SYR-SLN was determined in phosphate buffer (pH 7.4 and 6.8), using dialysis bag method. The dialysis bag has a molecular weight cut-off of 14000 and was soaked in double-distilled water for 12 h before use. Three mL of SYR-SLN dispersion (1 mg/ml) was transferred to the bag which was placed in a conical flask containing 30 mL of receiving medium. The conical flasks were placed in a thermostatic shaker (SHA-B, Constant-Temperature Shaker, China) at 37 ± 1 °C and 100 strokes/min. At predetermined time intervals, the medium in the flask was completely removed and 30 mL fresh medium was added to maintain the sink condition. The SYR content of the filtrate was determined by HPLC (n = 3).

3.7. Tissue distribution studies in rats

Animal experiments were carried out in accordance with the requirements of the National Act on the use of experimental animals (People's Republic of China). Sixty rats (20 ± 2 g, provided by Heilongjiang University of Chinese Medicine Animals Center) were used for our studies. All animals were fasted for 12 h before the experiments but had free access to water. The rats were randomly divided into two groups: SYR-SLN administration and SYR NS as control. A dose of 15 mg/kg was injected via the caudal vein and the animals and blood was subsequently taken at 5, 15, 30, 60 and 90 min by sampling blood from eyeball. Whole blood was then collected in heparinized tubes and immediately centrifuged (4000 rpm, 10 min) to obtain plasma. Heart, liver, spleen, kidneys and lung were also collected, cleaned with NS and then weighed. After adding predetermined amounts of NS, the organs were ground to give homogenates. The plasma and homogenates were stored at –20 °C for analysis.

Pharmacokinetics parameters are analysed by 3P97 program which was recommended by Chinese Pharmacologic Association. That is, re (relative targeting efficiency) and te (drug targeting efficiency). After intravenous

injection of SYR-SLN and syringopicroside NS, tissue concentrations of syringopicroside were determined using HPLC method. Syringopicroside accumulations in different tissues were calculated by trapezoidal rule, and then evaluated the distribution of syringopicroside in mice organs with drug targeting index and selective index. The amount of drug distribution in plasma and tissues was examined after intravenous administration of SYR-SLN and syringopicroside solution. Mice organization distribution experimental results show that the liver targeting efficiency of SYR-SLN is 1.65, and is much higher than any other organizations. The liver's te in nanoparticles group was also higher than the solution group's. So, syringopicroside is made into SYR-SLN which improved drug targeting effects and reached the target. SYR-SLN can make syringopicroside more concentrated at the lesion site, so SYR-SLN can improve curative effect.

Chromatographic conditions: The column was Diamonsil C_{18} (250 mm × 4.6 mm, 5 μm), the mobile phase consisted of methanol: purified water (50: 50, volume ratio). The flow rate and detection wavelength were 1.0 ml/min and 221 nm; column temperature: 30 °C.

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