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Studies on the cyclosporin A loaded stearic acid nanoparticles

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Abstract

Stearic acid nanoparticles were prepared in this study by melt-homogenization to investigate the possibility of them as a new kind of drug carrier system. Some physicochemical properties of stearic acid nanoparticles were studied and morphology examined by transmission electron microscope. Cyclosporin A as a model drug was then encapsulated into stearic acid nanoparticles. Following the establishment of high performance liquid chromatography assay for cyclosporin A analysis in stearic acid nanoparticles or blood samples, the encapsulation ratio of cyclosporin A to stearic acid nanoparticles was estimated and pharmacokinetics as well as bioavailability of cyclosporin A stearic acid nanoparticles after oral administration to Wistar rats were studied, using the Sandimmun Neoral® (an available microemulsion system of cyclosporin A) as a reference. The mean diameter of cyclosporin A stearic acid nanoparticles was 316.1 nm, while the encapsulation ratio of cyclosporin A to stearic acid nanoparticles reached to 88.36%. It was demonstrated by IR spectra and differential scanning calorimetry that there was no chemical reaction occurred between the cyclosporin A and stearic acid. The relative bioavailability of cyclosporin A stearic acid nanoparticles over reference was nearly 80%, and the time to reach maximum concentration (T_{max}) of cyclosporin A after oral administration of cyclosporin A stearic acid nanoparticles was delayed significantly than the reference, suggesting an obvious sustained release effect. The stearic acid nanoparticles might be a very potential drug carrier.

Keywords: Drug carrier; Stearic acid; Nanoparticles; Cyclosporin A; Relative bioavailability

1. Introduction

Particulate drug delivery system, such as nanoparticles and microspheres has been studied extensively in the past 10 years. But the toxicity of the carrier materials was still remained to be problems since the synthesized polymers such as alkylcyanoacrylate, poly(lactic acid), methylmethacrylate, and so on were often used. The possible accumulation and their toxic metabolic product were still worth to be further studied.

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In order to choice a better carrier material, an endogenous longchain saturated fatty acid (stearic acid) was studied in this investigation. The stearic acid is a main composition of fat, so it was expected to have better biocompatibility and lower toxicity than the synthesized polymers. Because it is solid at room temperature it was supposed to be more stable than liposome or emulsion. Stearic acid is available for pharmaceutical use and it is easy to prepare the nanoparticles with it. Several approaches on preparation (Schwarz and Wehnert, 1997; Freitas and Muller, 1998), stability (Heiati, et al., 1998; Freitas and Muller, 1999) and other aspects (Bargoni et al., 1998; Morel et al., 1998) have been reported recently about solid lipid nanoparticles.

The purpose of our present investigation was to evaluate the possibility of stearic acid nanoparticles as a new kind of drug carrier, through the studies of preparation procedure, physicochemical properties, quality as well as the pharmacokinetics and bioavailability of cyclosporin A (model drug) stearic acid nanoparticles after oral administration to rats.

2. Materials and methods

2.1. Materials

Cyclosprione A was obtained from Sichuan Industrial Institute of Antibiotics (Chengdu, China). Cyclosprione D was a gift from Northern-China Pharmaceutical Plant (Shijiazhuang, China). Stearic acid was the product of Shanghai People's Chemical Plant (Shanghai, China). Lecithin and Poloxomer were purchased from Sigma (St Louis, MO). Sandimmun Neoral® (microemulsion system of cyclosporin A) was the product of Sandoz (Sweden). Methanol and acetonitrile were purchased from Beijing People's Chemical Plant (Beijing, China). All other chemicals and solvents were of analytical grade.

2.2. Preparation ofstearic acid nanoparticles

Stearic acid nanoparticles were prepared by melt-homogenization (Siekmann and Westesen, 1994). Briefly, the aqueous solution containing surfactants was heated to 75°C, then under mechanical stirring the solution was added to stearic acid melted at the same temperature. The stirring was kept to form stearic acid nanoparticles until room temperature.

2.3. Physicochemical properties ofstearic acid nanoparticles

The morphology of stearic acid nanoparticles was examined by transmission electron microscope. Zeta potential was measured by an U-tube electrophoresis apparatus, surface tension by maximum bubble method, turbidity by recording the absorption at 600 nm, relative density by an areometer, viscosity by an Ubbelohde viscometer, pH value by a pH-25 acidimeter and refractive index by an Abbe refractometer.

2.4. Preparation of cyclosporin A stearic acid nanoparticles

Cyclosporin A stearic acid nanoparticles were obtained by the same approach mentioned above, except that cyclosporin A and stearic acid were melted together.

2.5. Encapsulation ratio of cyclosporin A to stearic acid nanoparticles

Cyclosporin A stearic acid nanoparticles were separated from the liquid medium by centrifuging (60 000 rpm, 2 h). The sediment obtained was dissolved in methanol, then analyzed at 55°C in a high performance liquid chromatography system with an Alltech C_{18} column (5 μm, 250 mm × 4.6 mm). The samples were eluted with methanol:water (90:10) at a flow rate of 1.0 ml/min and detected at 214 nm. Encapsulation ratio (ER) of cyclosporin A to stearic acid nanoparticles was calculated from: $ER = (W_m/W_a) \times 100\%$, where W_m represents the amount of cyclosporin A found while W_a means the amount of cyclosporin A added.

2.6. Particle size of cyclosporin A stearic acid nanoparticles

The colloidal solution of cyclosporin A stearic acid nanoparticles was diluted with distilled water, then analyzed in a particle size analyzer.

2.7. IR spectra and differential scanning calorimetry analysis

The IR spectra of cyclosporin A, freeze-dried stearic acid nanoparticles and freeze-dried cyclosporin A stearic acid nanoparticles were prepared in a IR spectrometer. The differential scanning calorimetry thermograms of stearic acid, the mixture of cyclosporin A and stearic acid, and the freeze-dried cyclosporin A stearic acid nanoparticles were made by a differential scanning calorimeter.

2.8. Analysis of cyclosporin A in blood samples

Cyclosporin A in blood samples was analyzed at 75°C by a high performance liquid chromatography system (Liang and Wu, 1992) with an Hypersil 120 C_{18} packed column (5 μm, 150 mm × 4.6 mm). The samples were eluted with acetonitrile:methanol:water (60:10:30) at a flow rate of 1.5 ml/min and detected at 210 nm. Cyclosporin D was taken as the internal material.

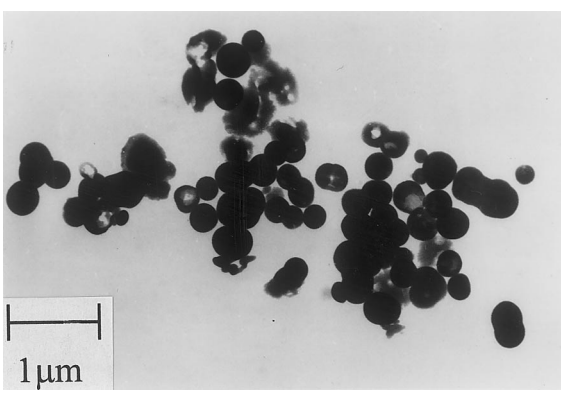

Fig. 1. Micrograph of stearic acid nanoparticles by transmission electron microscope (× 14 000).

2.9. Pharmacokinetics and bioavailability of cyclosporin A stearic acid nanoparticles in rats

Cyclosporin A stearic acid nanoparticles (the test) were compared with the reference, Sandimmun Neoral®. Ten 250 ± 25 g Wistar rats (Animal Center of Beijing Medical University), fasted overnight, were matched in pairs according to the body weight. After appropriate dilution, the single dose of cyclosporin A stearic acid nanoparticles or reference was given intragastrically to the rat in each pair, respectively. Venous blood were collected from the postorbital vein sinus before and over a 60-h period, then the plasma samples were separated and kept at 4°C until analysis.

The elimination rate constant (K_{el}) was calculated with log-linear regression, using the last three to five observations of each curve, and the elimination half-life ($t_{1/2}$) was derived from 0.693/K_{el}. The area under the plasma concentration–time curve (AUC_{0-t}) was calculated with the trapezoidal rule, and extrapolation of the area to infinity (AUC_{0-inf}) was estimated by adding the last measured plasma concentration divided by the elimination rate constant (K_{el}). A computer program 3P87 (Administration of Health, Beijing, China) was used for the fitting of pharmacokinetic models, and also for the calculation of the maximum plasma concentration (C_{max}) as well as the time to attain C_{max}(t_{max}). The relative bioavailability (Fr%) was calculated from (AUC_{0-inf} for 'test'/AUC_{0-inf} for 'reference') × 100%.

3. Results

The colloidal solution of stearic acid nanoparticles prepared was white and milk-like. Most of the nanoparticles looked round and regular under transmission electron microscope as shown in Fig. 1. Their physicochemical properties of stearic acid nanoparticles were listed in Table 1.

The stearic acid nanoparticles were negatively charged, but the zeta potential was rather low. The surface tension and pH value of stearic acid nanoparticles decreased, while the viscosity and turbidity increased, as the concentration of stearic acid nanoparticles increased. Little change was observed in relative density and refractive index.

Table 1
Physicochemical properties of stearic acid nanoparticles ($n = 5$)

Physicochemical properties	Concentration of SA-NP[a]		
	1.2%	2.0%	3.0%
Zeta potential (mV)	-3.45 ± 0.19	-2.60 ± 0.12	-4.02 ± 0.26
Surface tension (10^{-2}, N/m)	5.32 ± 0.09	4.75 ± 0.05	3.80 ± 0.04
Turbidity	0.037 ± 0.001	0.091 ± 0.002	0.2090 ± 0.001
Relative density (g/cm^3)	1.0015 ± 0.0001	1.0013 ± 0.0001	1.0016 ± 0.0002
Viscosity (10^{-3}, Ns/m^2)	1.404 ± 0.002	1.473 ± 0.003	1.933 ± 0.003
pH value	4.28 ± 0.06	3.93 ± 0.02	3.82 ± 0.02
Refractive index	1.3388 ± 0.0001	1.3389 ± 0.0001	1.3393 ± 0.0001

[a] SA-NP, stearic acid nanoparticles.

The encapsulation ratio of cyclosporin A to stearic acid reached to 88.36% as shown in Table 2, analyzed by HPLC method. The linear response of this assay ranged from 0.5 to 200 μg/ml. The analytical recoveries were 97.82, 100.03 and 100.71%, respectively, under three different concentration (5.0, 25.0 and 100.0 μg/ml), while the relative standard deviation within day were 0.54, 0.56 and 1.62%, respectively.

The mean diameter of cyclosporin A stearic acid nanoparticles was 316.1 nm, and 84.4% of the cyclosporin A stearic acid nanoparticles distributed between 176 and 297 nm.

It was demonstrated by IR spectra in Fig. 2 that there was no chemical reaction occurred between the cyclosporin A and stearic acid, because the characteristic peaks of cyclosporin A and stearic acid did not show any shifts after encapsulation of cyclosporin A to stearic acid. Studies on differential scanning calorimetry came to the same conclusion.

The baseline separation between cyclosporin A and cyclosporin D was achieved with the high performance liquid chromatography system established for analysis of cyclosporin A in blood samples. The standard curves were linear over the range of 50–1000 ng/ml, and the retention time of cyclosporin A and D were 6.96 and 9.16 min, respectively. The detective limitation was 20 ng/ml. The analytical recoveries were 111.7, 101.1 and 100.8%, respectively under three different concentration (50, 300 and 1000 ng/ml), while the relative standard deviation within day were 5.3, 3.6 and 2.7%, respectively.

Cyclosporin A plasma concentrations (mean ± S.D.) for both single dose oral administration of cyclosporin A stearic acid nanoparticles and the reference were shown in Fig. 3, and the derived pharmacokinetic parameters in Table 3.

A rapid increase followed by a rapid decrease in cyclosporin A plasma concentrations resulted after oral administration of microemulsion system

Table 2
Encapsulation ratio of cyclosporin A to stearic acid nanoparticles ($n = 3$)

No.	CyA[a] added (μg/ml)	CyA found (μg/ml)	Encapsulation ratio	
			%	Mean %
1	27.85	24.67	88.69	88.36 ± 2.19
2	26.38	22.74	86.28	
3	27.45	24.64	90.11	

[a] CyA, cyclosporin A.

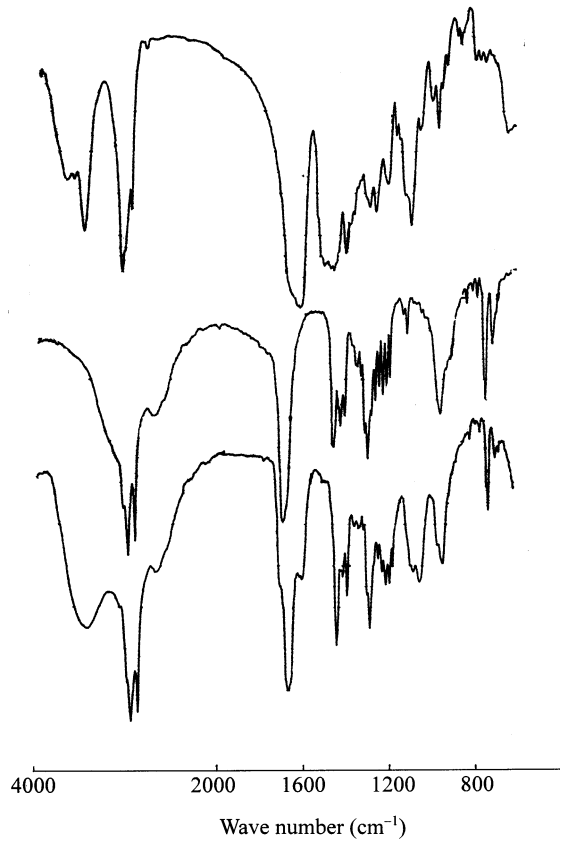

Fig. 2. The IR spectra of cyclosporin A (upper), stearic acid (middle) and cyclosporin A stearic acid nanoparticles (down).

Table 3
Pharmacokinetic parameters of cyclosporin A after single dose oral administration of cyclosporin A stearic acid nanoparticles or Sandimmun Neoral® to Wistar rats

Pharmacokinetic parameter	CyA–SA-NP[a] (1.2325 mg/100 g)	Sandimmun Neoral® (1.0 mg/100 g)
C_{max} (ng/ml)	1033.1 ± 353.2	1736.4 ± 358.0
t_{max} (h)	4.5 ± 2.2	1.75 ± 0.43
AUC_{0-60} (μg h/ml)	22.77 ± 3.36	23.27 ± 3.43
AUC_{0-inf} (μg h/ml)	25.04 ± 3.16	25.47 ± 3.26
K_{el} (1/h)	0.0453 ± 0.0122	0.0464 ± 0.0177
$T_{1/2}$ (h)	16.42 ± 4.16	16.90 ± 5.29
Fr (%)	79.77	

[a] CyA–SA-NP, cyclosporin A stearic acid nanoparticles.

of cyclosporin A, with C_{max} occurring at about 1.7 h. Relatively slow increase and also slow decrease in cyclosporin A plasma concentrations were observed after administration of cyclosporin A stearic acid nanoparticles, with the C_{max} of cyclosporin A occurring at 4.0 h which is delayed significantly ($P < 0.05$) than the reference, suggesting an obvious sustained release effect of it. The pharmacokinetic parameters such as K_{el} and $t_{1/2}$ shown no statistically significant difference ($P > 0.05$) between the two treatments.

The relative bioavailability of cyclosporin A stearic acid nanoparticles over reference was

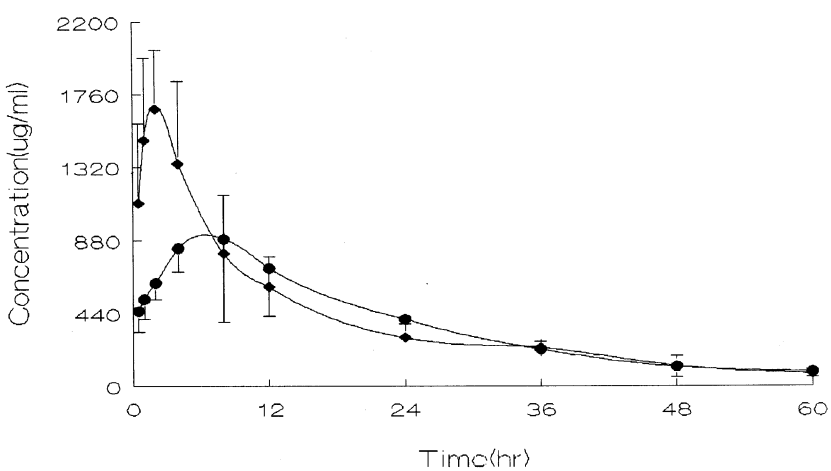

Fig. 3. Mean plasma concentration of cyclosporin A vs. time curves after single oral administration of cyclosporin A stearic acid nanoparticles (●) or Sandimmun Neoral® (◆) to rats.

nearly 80%, indicating that stearic acid nanoparticles could improve the absorption of some insoluble drug, such as cyclosporin A, in the gastrointestinal tract. The cyclosporin A plasma concentration–time curve after single oral administration of cyclosporin A stearic acid nanoparticles was well described by biexponential equation according to a one-compartment open model, while the cyclosporin A plasma concentration–time data was well fitted by a two-compartment model with one-order absorption kinetics.

4. Discussion

The preparation techniques investigated in this work included solvent-evaporation and melt-homogenization, and the sonication dispersion and mechanical dispersion had been used in melthomogenization. The stearic acid nanoparticles prepared by solvent-evaporation could be very small and unique but always aggregated to form precipitation, while the nanoparticles obtained by sonication dispersion at high temperature were irregular and rather big in diameter. Mechanical dispersion from high temperature to room temperature was proved to be the best method in the preparation of stearic acid nanoparticles. Small, unique and stable tearic acid nanoparticles could be prepared with this technique. Effects of stirring time, stirring speed and temperature on the particle size and morphology were studied and the best formulation was selected. It was demonstrated that stirring time and speed show no obvious affects on the particle size and its homogeneity, and the present of cyclosporin A had no influence on the formation of nanoparticles.

It was observed in the established HPLC system that the temperature show great impact on the column efficiency, and the column efficiency increased with the rise of temperature. A temperature of 55°C was selected for the analysis temperature for analysis of cyclosporin A in stearic acid nanoparticles in consideration of column damage at high temperature. But higher temperature was employed for analysis of cyclosporin A in blood samples because more impurities existed and baseline separation of cyclosporin A and D was needed.

The Sandimmun Neoral®, an available microemulsion system of cyclosporin A was used as a reference in the studies of pharmacokinetics and bioavailability of cyclosporin A stearic acid nanoparticles after oral administration. As a new product of Sandoz, the microemulsion system, made of surfactants and oil phase, can form emulsion droplets only about 30 nm whenever it contact with water, so it is specially advantageous for the absorption of cyclosporin A, which is a typical poor soluble drug. Even compared with such formulation, the relative bioavailability of cyclosporin A stearic acid nanoparticles was nearly 80%, indicated that stearic acid nanoparticles could improve the absorption of cyclosporin A. The major reason of lower bioavailability of cyclosporin A stearic acid nanoparticles might be the size of the nanoparticles (316.1 nm) which is tenfold of the microemulsion droplets (≈ 30 nm), and other possibility may due to the different surfactants used.

It was noticed in Fig. 3 that the profiles of the cyclosporin A plasma concentrations after single dose oral administration of cyclosporin A stearic acid nanoparticles and Sandimmun Neoral® were quite different. It could be attributed to the different release rate of different delivery systems, because the insoluble cyclosporin A is rather ready to release from the liquid microemulsion droplets than from the solid nanoparticles.

The effect of food and particle size on the absorption of cyclosporin A in GI tract was remained to be further studied.

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