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Pharmacokinetic behavior of cyclosporin A in rabbits by oral administration of lecithin vesicle and sandimmun neoral

Jianxin Guo, Qineng Ping *, Ying Chen

Department of Pharmaceutics, China Pharmaceutical University, Nanjing 210009, People's Republic of China

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Abstract

The present study was undertaken to investigate the incorporation of lipophilic polypeptide, cyclosporin A (CsA) into lecithin vesicular system and to compare its pharmacokinetics behavior with Sandimmun Neoral® (CsA-NEO). Lecithin vesicles of cyclosporin A (CsA-VES) were prepared by the rotary evaporation method, treated further with sonication. Studies were carried out to characterize the vesicles on physical properties, content, entrapment efficiency, particle size, polydispersity and Zeta potential. Pharmacokinetic behaviors were studied in rabbits at dose of 30 mg/kg. Results showed CyA vesicles were spherical particles, with content of $3.137 \pm 0.060\%$ mg/ml, entrapment efficiency of $98.91 \pm 0.80\%$, particle size of 63.89 ± 4.75 nm, polydispersity of $43.2 \pm 6.1\%$ and Zeta potential of -13 mV. The best model fitting experimental data was a two-compartment open model with first-order kinetics. The relative bioavailability of CsA-VES versus CsA-NEO was $105 \pm 21\%$ ($n = 6$) and statistical analysis demonstrated both preparations were bioequivalent. In conclusion, lecithin vesicles are promising carriers in the oral delivery of CsA, considering their absorption enhancement effect and low-toxic property.

Keywords: Lecithin vesicles; Microemulsion; Cyclosporin A; Pharmacokinetics; Relative bioavailability

1. Introduction

Nowadays, numerous efforts are being made to enhance the oral bioavailability of polypeptides in order to increase their clinical efficacy. The incorporation of the active component into lipid vesicles is very attractive. Advantages include increased solubility and protection of the polypeptides from the aggressive conditions present in the gastrointestinal tract (Arien et al., 1994, 1995). Furthermore, the biomembrane similarity of the lecithin material and the small particle size of the vesicles may result in rapid absorption (Aungust, 1993). It has been reported that oral administration of liposomes loaded with calcitonin led to hypocalcemic effect in the rat 1 h after the application (Fukunaga et al., 1991).

* Corresponding author. Tel.: +86-25-3301606; fax: +86-25-3302827.

E-mail address: pingqn@mailbox.cpu.edu.cn (Q. Ping).

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Cyclosporin A (CsA) is a lipophilic cyclic polypeptide containing 11 amino acids and was reported as a typical drug with the worst and non-regular absorption (Klompmaker et al., 1993). The present study was undertaken to characterize the incorporation of CsA into lecithin vesicular system and to compare its pharmacokinetics behavior in rabbits with Sandiummun Neoral® (CsA-NEO). Sandiummun Neoral® is a highly recommended new microemulsion formulation concentrated CsA (Kovarik et al., 1994).

2. Materials and methods

2.1. Materials

Soybean lipid (purity $>80\%$, lot number: 980607) was purchased from Shanghai Fuda pharmaceutical manufacturing factory (China) and lecithin was freshly prepared by column chromatography on aluminum oxide according to the reported method (Singelton et al., 1964). Only lecithin with purity $>95\%$ was used in this study. CsA powder (USP 23, lot number: 040396) was obtained from Galena (Czech Republic). Methanol, chloroform, sodium chloride and other reagents, all p.a. were products of Nanjing Chemical Corporation (China).

2.2. Preparation of lecithin vesicles

Lecithin vesicles of CsA (CsA-VES) were prepared by conventional rotary evaporation–sonication method. Appropriate amounts of lecithin (4 wt.%) and CsA (0.375 wt.%) were dissolved in co-solvent of methanol and chloroform (1:1). The mixture was dried to a thin film under vacuum. The film was then hydrated with 0.9% NaCl solution to make a lipid coarse suspension. Sonication was carried out at 4°C (JY 92-II ultrasonic processor, China) to obtain small vesicles.

2.3. Characterization of vesicles

Diameter, polydispersity (V) and Zeta potential of vesicles was measured by a Zetamaster 'S' instrument (Malvern Instruments, Malvern, UK).

Samples for transmission electron microscopy (TEM) were prepared at room temperature by conventional negative staining methods using 1% phosphotungstic acid buffer (pH 6). Samples were viewed on an H-7000 model, transmission electron microscope (Hitachi, Japan).

2.4. Content and entrapment efficiency determination

Concentration of CsA was determined by high-pressure liquid chromatography (HPLC). The HPLC system consisted of a pump (Model LC-5A, Shimadzu, Japan), a shim-pack CLC-ODS column (150×6 mm ID, Shimadzu) maintained at 70°C, an UV detector (Model SPD-6A, Shimadzu, Japan) at 210 nm and a data station (Model C-R6A, Shimadzu, Japan). The mobile phase was composed of 72% (v/v) methanol and 28% water, and delivered at a flow rate of 1.2 ml/min. The injection volume was 20 μl and the relative retention time was found to be 11 min.

Content determination was carried out by dissolving both preparations in absolute ethanol and measured by HPLC.

Separation of free drug and vesicles was effected by passing through Sephedex-G50 (16×3 cm) column, then eluted by distilled water with speed of 0.5 ml/min. Content of free drug was determined by HPLC. The entrapment efficiency was calculated as the ratio of drug weight within the vesicles to the content of drug in the vesicular suspension.

2.5. Pharmacokinetics study

2.5.1. Study design

A complete crossover design was carried out with an interval of 1 week. Two groups of rabbits (weighed 1.8–2 kg, provided by Central Animal Laboratory of China Pharmaceutical University) were fasted overnight and randomized to receive CsA-NEO and CsA-VES at a dose of 30 mg/kg. Blood samples were obtained at just prior to and 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12 and 24 h following oral administration.

2.5.2. Extraction of CsA

Blood samples were extracted with 2.5 ml of ether and 2.5 ml of hexane. After shaking for 5 min and centrifugating for 5 min at 2000 rpm, the organic phase was transferred to a 5 ml conical tube for evaporation until dryness, under a stream of nitrogen at 35°C. Residue was re-dissolved in 100 μl of the mobile phase and 150 μl of hexane, then it was shaken for 1 min and centrifuged for 5 min at 2000 rpm. An aliquot of 20 μl was injected into the HPLC.

2.6. Data analysis

2.6.1. Pharmacokinetics analysis

The pharmacokinetic parameters associated with each rabbit were estimated by compartmental and non-compartmental methods. Nonlinear regression analysis showed that the best model fitting experimental data was a two-compartment open model with first-order input and first-order output from the central compartment. The corresponding pharmacokinetic parameters were derived by pharmacokinetics analysis program.

On the basis of non-compartmental analysis, statistical moments were determined. The zero-order moment area under the curve (AUC_T) was determined to the last experimentally measured concentration $C_n(t_z)$ at time t_z by the trapezoidal rule and extrapolated to infinity by adding the term $C_n(t_z)/\lambda_z$, where λ_z was the slope of the terminal phase estimated by log-linear regression of the last four to five experimental blood concentrations. The mean residence time (MRT) was derived from the ratio $AUMC_T/AUC_T$, where $AUMC_T$ was the area under the curve for the plot of the product of concentration and time versus the time from time zero to infinity.

2.6.2. Statistical analysis

A paired t-test was applied on differences in bioavailability, peak level, time that peak level reached (t_{peak}) and MRT. ln(AUC) and ln(C_{max}) were compared by using two-sided t-test and analysis of variance (two way ANOVA) at the 0.05 significance level (SAS software package).

3. Result

3.1. Physical properties of vesicles

CsA-VES suspension was transparent colloidal dispersions with average diameter of 77.6 nm and the polydispersity index of 45.6%. Zeta potential was -13 mV.

Fig. 1 showed the transmission electron photomicrographs of vesicles. It was evident that the particles were approximately spherical with obvious whorls.

3.2. Determination of CsA

The regression equation for CsA content (μg/ml) in ethanol solution ranging from 0.1 to 4 μg/ml was $A = 7.52 \times 10^3 C - 74.66$ ($r^2 = 0.9999$). The mean recovery was $97.4 \pm 5.9\%$ ($n = 3$). Precision assay showed the average of the relative standard deviations (S.D.) within 1 day was 1.5% and among every other day was 2.0%.

The regression equation for CsA content (ng/ml) in the blood ranging from 20 to 2000 ng/ml was $A = 54.32C + 249.68$ ($r^2 = 0.9999$). The mean recovery was $97.0 \pm 7.4\%$. Precision assay showed that the average of the relative standard deviations (R.S.D.) within 1 day was 3.4% and among every other day was 5.0%.

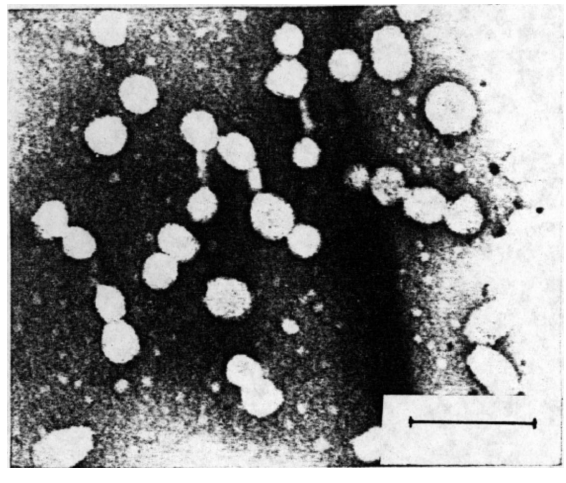

Fig. 1. Electron photomicrographs of lecithin vesicles containing cyclosporin A (magnification $\times 180\,000$). Bar is 100 nm.

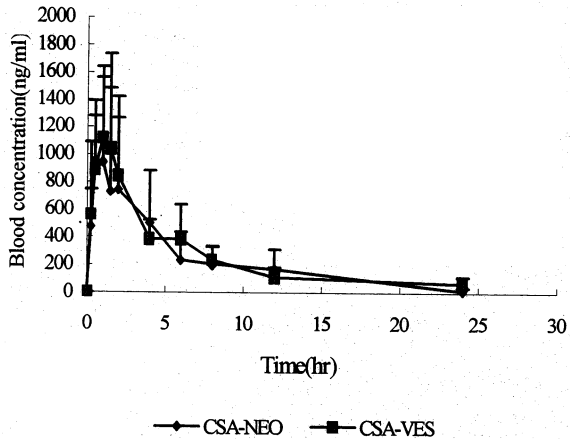

Fig. 2. Mean ± S.D. whole blood concentration-time profiles of rabbits following oral administration of CSA-VES and CSA-NEO ($n = 6$).

3.3. Content and entrapment efficiency of CsA-VES

The content of CsA-VES was 3.137 ± 0.060%.

Column recoveries of free drug and blank vesicles were 98.89 ± 2.14 and 99.6 ± 2.68%, respectively. The elution volume of blank vesicles was 17–25 ml and that of CsA was 28–46 ml. Vesicles and CsA were separated at 25 ml. Partly due to the lipophilic nature of CsA and the strong affinity for lipid, the entrapment efficiencies of vesicles were high, upto 98.97 ± 0.08%.

3.4. Pharmacokinetics

The blood CsA concentration-time profiles during the course of study were shown in Fig. 2. Non-linear fitting of experimental data to the two-compartment model generated the pharmacokinetic parameters presented in Table 1. An adequate data fit was evident by the high correlation ($r^2 > 0.99$) between computer-calculated and experimental CsA blood concentrations and the plot of residuals. The inter-compartmental drug distribution rates (K_{12} and K_{21}) and elimination rates did not show statistical significant differences between both preparations ($P > 0.05$).

The mean pharmacokinetic parameters derived from a non-compartmental analysis were presented in Table 2. A mean peak plasma concentration of about 1200 ng/ml was reached at approximately 0.8–1 h for both formulations. The relative bioavailability of CsA-VES versus CsA-NEO was 105 ± 21%. Paired t-test showed there were no significant differences in peak concentrations, t_{peak}, MRT and AUC ($P > 0.05$). Similarly, two-sided t-test and ANOVA indicated there were no significant differences in ln(AUC) and ln(C_{max}). Statistical analysis demonstrated both preparation were bioequivalent.

4. Discussion

CsA is a potent immunosuppressive drug and has been utilized clinically in the prevention of organ rejection (Mcevoy, 1995). Due to its high lipophilicity, poor solubility in aqueous fluids, the existence of an absorption window in the small intestine (Reymond et al., 1988) and relatively high molecular weight, the mean absolute bioavailability of CsA is approximately only 30%, when the drug is administered as Sandiummun®, a crude oil-in-water emulsion (Mcevoy, 1995). In order to increase bioavailability, a new microemulsion pre-concentrate formulation, Sandiummun Neoral®, has been developed. This new formulation has been shown to increase bioavailability by 40–60% in comparison with Sandiummun® (Kovarik et al., 1994).

The incorporation of CsA in the vesicles increased drug solubility, the entrapment efficiency

Table 1
Pharmacokinetic parameters of CsA preparations ($n = 6$)

	Ka	K_{21}	K_{10}	K_{12}	α	$t_{1/2\alpha}$	β	$T_{1/2\beta}$
CsA-NEO	1.68 ± 0.78	0.25 ± 0.09	0.33 ± 0.13	0.67 ± 0.30	1.18 ± 0.29	0.63 ± 0.13	0.07 ± 0.03	12.28 ± 4.69
CsA-VES	1.68 ± 0.77	0.41 ± 0.56	0.40 ± 0.16	0.44 ± 0.29	1.18 ± 0.35	0.65 ± 0.18	0.06 ± 0.02	14.13 ± 5.63

Table 2
Non-compartment analysis and some moment parameters of CsA preparations

	T_p (h)	C_{max} (ng/ml)	AUC_{0-24} (ng/ml per h)	$AUC_{0-\infty}$ (ng/ml per h)	AUMC (ng/ml per h^2)	MRT (h)	V (l/kg)	λ_Z (h)
CsA-NEO	1.08 ± 0.53	1132 ± 542	5659 ± 3688	5769 ± 3761	$135\,131 \pm 163\,082$	15.15 ± 8.98	12.50 ± 5.07	0.33 ± 0.13
CsA-VES	0.83 ± 0.78	1299 ± 442	6076 ± 2780	6318 ± 2914	$161\,856 \pm 126\,748$	14.92 ± 6.09	13.66 ± 4.65	0.40 ± 0.16

was high, which facilitated drug penetration through the gastrointestinal mucin layer (Gershanik et al., 1998). The small vesicular size (< 100 nm) increased the available surface area for drug partitioning, promoting the uptake by Peyer's patches (Sass et al., 1990). Furthermore, lecithin, as component of cell membrane, may enhance neutralization, thus increase the absorption by lymph-circulation (Yanagawa et al., 1989).

In the present study, lecithin vesicular carrier has enhanced the absorption of CsA and CsA-vesicles were bioequivalent with the Sandiummun-Neoral®. On the other hand, lecithin vesicles made from biological phospholipids are biodegradable, relative lack of immunogenicity and exhibit low intrinsic toxicity (Vadiei et al., 1989). Such properties are advantageous in the delivery of drugs.

In conclusion, lecithin vesicles are promising carriers in the oral delivery of CsA, considering their absorption enhancement effect and low-toxic property.

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