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Bioavailability of cyclosporin A dispersed in sodium lauryl sulfate–dextrin based solid microspheres

Eun-Jin Lee, Sa-Won Lee, Han-Gon Choi [1], Chong-Kook Kim *

National Research Laboratory for Drug and Gene Delivery, College of Pharmacy, Seoul National University, San 56-1, Shinlim-Dong, Kwanak-Ku, Seoul 151-742, South Korea

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Abstract

The purpose of this work was to develop a solid dispersion system containing cyclosporin A (CsA) in order to improve the bioavailability of poorly water-soluble CsA. Solid dispersion systems that are spherical in shape (CsA–microspheres) were prepared with varying ratios of CsA/sodium lauryl sulfate/dextrin using a spray-drying technique. The effects of sodium lauryl sulfate (SLS) and dextrin on the dissolution of CsA dispersed in SLS–dextrin based solid microspheres were investigated. The bioavailability of CsA–microspheres was compared with CsA powder alone and commercial Sandimmun® in dogs. SLS significantly enhanced the dissolution of CsA from microspheres, while dextrin did not affect this. The CsA–microspheres at the CsA/SLS/dextrin ratio of 1/3/1, which gave the highest dissolution rate of CsA among the formula treated, was selected as an optimal formula for oral delivery. This formula gave significantly higher blood levels, area under the drug concentration-time curve (AUC) and maximum blood concentration of drug (C_{max}) of CsA in dogs compared with the CsA powder alone. The AUC, C_{max} and time to reach maximum blood concentration (T_{max}) of CsA with CsA–microspheres was not significantly different from those after oral administration of Sandimmun®, suggesting the similar bioavailability to Sandimmun® in dogs. Our study demonstrates that the CsA–microspheres prepared with SLS and dextrin, with improved bioavailability of CsA, would be useful to deliver a poorly water-soluble CsA and could be applicable to other poorly water-soluble drugs.

Keywords: Bioavailability; Cyclosporin A; Dextrin; Microsphere; Sodium lauryl sulfate; Solid dispersion

1. Introduction

Cyclosporin A (CsA), a cyclic oligopeptide, has been extensively used as an immunosuppressant in organ transplant patients (Cohen et al., 1984) and for the treatment of autoimmune diseases (Beveridge, 1983; Thomson and Neild, 1991). However, it is known that the oral bioavailability of CsA is

* Corresponding author. Tel.: +82-2-8807867; fax: +82-2-8737482.

E-mail address: ckkim@plaza.snu.ac.kr (C.-K. Kim).

[1] Present address: College of Pharmacy, Yeungman University, 214-1, Dae-Dong, Gyongsan 712-749, South Korea.

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usually very low due to the poor absorption, which is related to the relatively high molecular weight, very high lipophilicity (log $P = 2.92$, Taylor et al., 1993) and poor solubility in aqueous medium (Ismailos et al., 1991). Various oral formulations of CsA such as a microemulsion (Drewe et al., 1992; Gao et al., 1998; Kim et al., 1997; Tejani, 1998), complexation with cyclodextrin (Miyake et al., 1999) and microspheres (Chacon et al., 1999; Urata et al., 1999) have been developed to enhance the solubility, dissolution rate and bioavailability of CsA.

Solid dispersion systems have been used as a formulation for improving the solubility, release and bioavailability of poorly water-soluble drugs. Polymers have been generally used as carriers of solid dispersion systems. The solid dispersion systems with polymers such as Eudragit® (Otsuka et al., 1993), sodium carboxymethylcellulose (Ubaldo et al., 1991), hydroxypropylcellulose (Yuasa et al., 1993) and chitosan and ethylcellulose (Dangprasirt and Pongwai, 1998), improved the solubility of drugs, but usually retarded their release. The solid dispersion systems with hydrophilic or amphiphilic polymers such as polyethylene glycol (Owusu-Ababio et al., 1998), phospholipid (Habib et al., 1998) and isopropyl myristate (Wolf, 1998) improved the solubility, dissolution and absorption of poorly water-soluble drugs. However, they were not suitable for solid dosage form, since they were relatively soft and not free-flowing. In the worst case, they were semisolid and waxy (Serajuddin, 1999). The solid dispersion systems with dextrin derivatives (Palmieri et al., 1998; Te Wierik et al., 1994) improved the solubility, dissolution and absorption of drugs, and were suitable for solid dosage form due to their free-flowing property. SLS was also used as a solubilizer or co-carrier of solid dispersion systems to improve the solubility and dissolution rate of drugs, and flow characteristics of powder (Ghosh et al., 1998; Khanfar et al., 1997). Thus, in the formulation of CsA solid dispersion systems suitable for solid dosage form, water-soluble dextrin and sodium lauryl sulfate (SLS) were used as a carrier and solubilizer, respectively.

To develop a CsA solid dispersion system for improving the bioavailability of CsA, dextrin–SLS based microspheres containing CsA were prepared with varying ratios of CsA/SLS/dextrin using a spray-dryer (Choi and Kim, 2000). The effects of SLS and dextrin on the dissolution rate of CsA were investigated. The bioavailability of CsA–microspheres was compared with CsA powder alone and commercial Sandimmun® in dogs.

2. Materials and methods

2.1. Materials

CsA and dextrin (TK-16) were supplied by Re-Yon Pharmaceutical Co. (Seoul, Korea) and Matsdani Chemical Co. (Tokyo, Japan), respectively. SLS was purchased from Aldrich Chemical Co. (Milwaukee, WI). Ethanol was of food grade. All other chemicals were of reagent grade and used without further purification.

2.2. Preparation of CsA-microspheres

Dextrin–SLS based CsA–microspheres were prepared using a nozzle type spray dryer (Model 190, Büchi, Flawil, Switzerland). CsA (1g) and a varying amount of SLS and dextrin, as shown in Table 1, were dissolved in 80 ml of 1:1 mixture of ethanol–water. This solution was kept at 60°C and delivered to the nozzle at a flow rate of 5 ml/min using a peristaltic pump and thereafter spray-dried at 120°C inlet and 75°C outlet temperatures. The pressure of spray air was 3 kg/cm^2 and the flow rate of dry air was about 25 mb. The direction of air flow was the same as that of

Table 1
Compositions of CsA-microspheres at the ratio of CsA/SLS/dextrin

Formula Ingredients	I	II	III	IV	V	VI	VII
CsA	1	1	1	1	1	1	1
SLS	0	0.7	1.5	3	5	3	3
Dextrin	4	4	4	4	4	5	1

sprayed product (Kim et al., 1994; Lee et al., 1998). The overall yield of CsA–microspheres obtained after spray-drying was approximately 70%.

2.3. Shapes and size of CsA–microspheres

The shape and surfaces of CsA–microspheres were examined using a scanning electron microscope (JEOL, JJM-35, Tokyo, Japan). The CsA–microspheres were loaded on the specimen stub via double-sided sticky tape and coated with gold(JEOL, Fine Coater, Tokyo, Japan) for 20 min at 100–200 mTorr in a shutter coater before taking photographs at an accelerating voltage of 2.4 kV.

A laser particle analyser (Helium-neon laser, wavelength 632.8 nm and radiant power 5 mW, Fritch Co., Germany) was used to measure the mean particle size and the size distribution of CsA–microspheres. The volume percent data over the particle diameter ranging from 1 to 250 μm was recorded.

2.4. Dissolution studies

CsA powder alone and CsA–microspheres, equivalent to 25 mg of CsA, were filled in gelatin hard capsules, respectively. Each gelatin hard capsule was put into a sinker in the dissolution apparatus. The dissolution studies were performed in 500 ml of 0.1 N HCl/acetonitrile (7:3, volume ratio) at 37 ± 0.5°C with stirring speed at 100 rpm. At predetermined time intervals, 1 ml of the medium was sampled, filtered through a membrane filter (0.45 μm) and determined using HPLC (Waters, Model TM 717) equipped with a μ-Bondapak C_{18} column (Waters, 0.5 μm, 25 × 0.46 cm i.d.) and UV detector (Model SPD-6A). The mobile phase consisted of water and acetonitrile (4:6, volume ratio). The eluent was monitored at 210 nm with a flow rate of 1.7 ml/min (Kim et al., 1997).

2.5. Pharmacokinetic studies

All experiments were performed according to the Seoul National University guideline of experimental animal care.

2.5.1. Treatment groups

Male beagle dogs weighing 12.6 ± 1.5 kg were fasted overnight prior to the experiments, but allowed free access to water. Twelve dogs were divided into three groups.

2.5.2. Administration and blood-collecting

CsA powder-filled gelatin hard capsules, CsA–microsphere-filled gelatin hard capsules and Sandimmun® (equivalent to 7.6 mg of CsA/kg) were orally administered to dogs in each group, respectively. About 10 ml of water was administered immediately with a syringe into the mouth. The muzzle was then clasped, so that the dog swallowed it. Blood samples were withdrawn at designated time intervals by puncture in the cephalic vein and frozen with 30 μl of 3% EDTA solution until analysis.

2.5.3. Blood sample analysis

CsA concentration in whole blood was measured by the radioimmunoassay (RIA) method using CYCLO-Trac™ SP-Whole Blood® RIA kit (INCSTAR Corporation, Stillwater, MN). The whole blood samples were extracted with methanol, vortexed for 15 s and centrifuged at $1600 \times g$ at room temperature for 5 min. Fifty microliters of methanolic supernatant were withdrawn, and incubated with 100 μl of ^{125}I ligand and 1 ml of pre-mixed antibody solution for 1 h. After centrifugation at $1600 \times g$ for 20 min, the supernatant was immediately decanted and the radioactivity of the precipitate of each tube was determined using γ-scintillation counter (Gao et al., 1998).

2.5.4. Pharmacokinetic data analysis

The area under the drug concentration–time curve (AUC) was calculated using the trapezoidal rule (Gibaldi and Perrier, 1982). The maximum blood concentration of drug (C_{max}) and time to reach maximum blood concentration (T_{max}) were obtained from blood data. Levels of statistical significance ($P < 0.05$) were assessed using the Duncan method of ANOVA. All results were expressed as mean ± standard deviation.

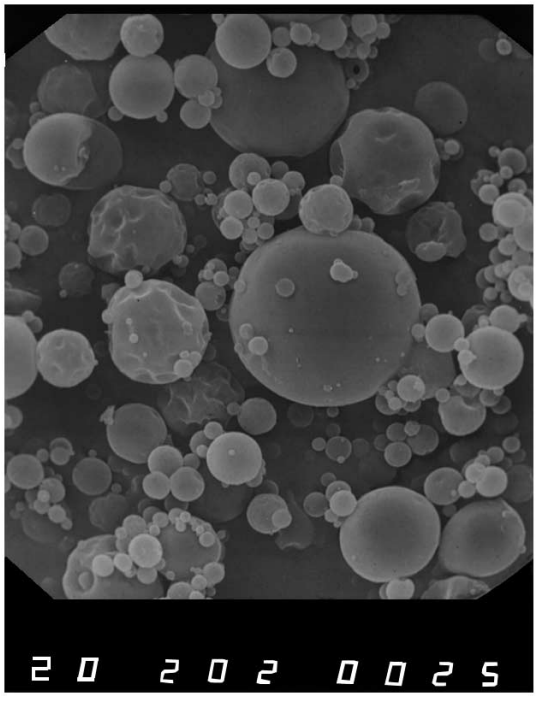

Fig. 1. Representative scanning electron micrograph of CsA–microspheres (3000 ×).

3. Results and discussions

3.1. Shapes and size of CsA–microspheres

CsA–microspheres, CsA solid dispersion systems, were prepared with varying ratios of CsA/SLS/dextrin using a spray-dryer. The compositions of the solid dispersion systems are summarized in Table 1. The scanning electron micrograph of the CsA–microspheres showed that the major particles were spherical in shape with a smooth surface as shown in Fig. 1.

The log normal number distribution of particle size is commonly used to predict the geometric mean diameter and geometric standard deviation from a linear relationship between the logarithm of the particle size and the cumulative percent frequency on a probability scale (Kim and Yoon, 1995). The log–probability plots of number-based size distribution of CsA–microspheres are given in Fig. 2. The geometric mean diameters of the CsA–microspheres at the CsA/SLS/dextrin ratio of 1/3/4 (formula IV), 1/3/5 (formula VI) and 1/3/1 (formula VII) were 3.21 ± 0.56, 3.22 ± 0.75 and 2.53 ± 0.92 μm, respectively. These data indicate that the geometric mean diameters of CsA–microspheres were not significantly affected by the ratio of CsA/SLS/dextrin.

3.2. Dissolution of CsA in dextrin–SLS based microspheres

CsA had a very low dissolution rate in an aqueous medium due to its very low water-solubility. In this case, a proper surfactant or organic solvent might be added to the dissolution medium in order to accelerate the dissolution test (Abdou, 1989). Since the CsA–microspheres in this study were prepared with the surfactant (SLS), addition of organic solvent was considered for the dissolution test. A mixture of 0.1 N HCl and acetonitrile (7:3, volume ratio) was selected as a dissolution medium based on the preliminary studies.

Fig. 3 describes the effect of SLS on the dissolution of CsA loaded in CsA–microspheres prepared with various ratios of CsA/SLS/dextrin. The dissolution rate of CsA loaded in micro-

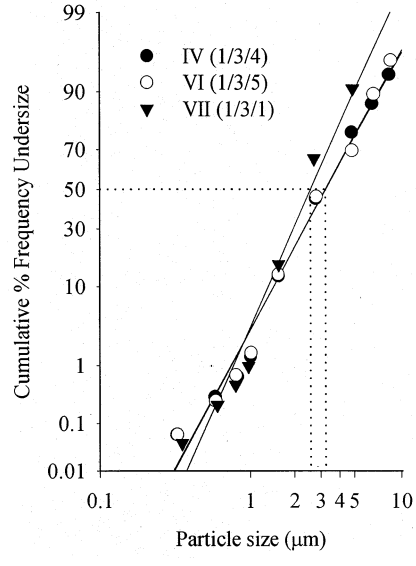

Fig. 2. Log–probability plots of number-based size distributions of CsA–microspheres at the CsA/SLS/dextrin ratio of 1/3/4 (formula IV), 1/3/5 (formula VI) and 1/3/1 (formula VII).

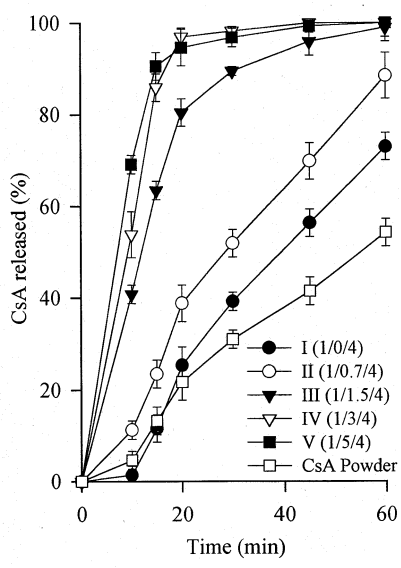

Fig. 3. Effect of SLS on the dissolution of CsA loaded in CsA–microspheres prepared with different ratios of CsA/SLS/dextrin. Data are expressed as mean ± S.D. ($n = 6$).

spheres prepared with dextrin alone (formula I), was slightly higher than that of CsA powder alone, indicating that the dextrin-based solid dispersion system improved the dissolution rate of CsA. The dissolution rates of CsA loaded in CsA–microspheres with SLS (formula II-V), further increased compared with that without SLS (formula I). The initial dissolution rates of CsA loaded in CsA–microspheres significantly increased according to the increase in the SLS/CsA up to 1.5/1 (formula I–III). At above a 1.5/1 ratio of SLS/CsA, the dissolution rate was not significantly increased (formula III–V). This result indicates that the inclusion of SLS at a 3/1 ratio of SLS/CsA is needed to maximize the dissolution rate of CsA loaded in microspheres. Additionally, the dissolved amount of CsA from CsA powder alone and CsA–microspheres at a SLS/CsA ratio of 3/1 (formula IV) was about 50 and 100% within 60 min, respectively. It indicates that the dissolved amount of CsA loaded in CsA–microspheres (formula IV) increased two-fold compared with that of CsA powder alone.

Fig. 4 illustrates the effect of dextrin on the dissolution of CsA loaded in CsA–microspheres prepared with various ratios of CsA/SLS/dextrin. When the dextrin/CsA ratio in CsA–microspheres changed from 1/1 to 5/1, the dissolution rates of CsA were similar to one another, suggesting that dextrin hardly affected the dissolution rate of CsA loaded in CsA–microspheres in the presence of SLS. Our results are in accordance with previous studies demonstrating that SLS had a greater effect on the dissolution of CsA than did dextrin (Palmieri et al., 1998; Te Wierik et al., 1994). However, the CsA–microspheres prepared with SLS alone were not suitable for the preparation of the solid dosage form, since they were relatively soft and not free-flowing (Serajuddin, 1999). Thus, the CsA–microspheres at the CsA/SLS/dextrin ratio of 1/3/1 (formula VII), which gave the highest dissolution rate and was suitable for preparing CsA–microspheres, was selected as an optimal formula of CsA for the oral delivery system in the subsequent study.

3.3. Pharmacokinetic analysis

The pharmacokinetic parameters of CsA were determined after oral administration of CsA–microspheres at a CsA/SLS/dextrin ratio of 1/3/1

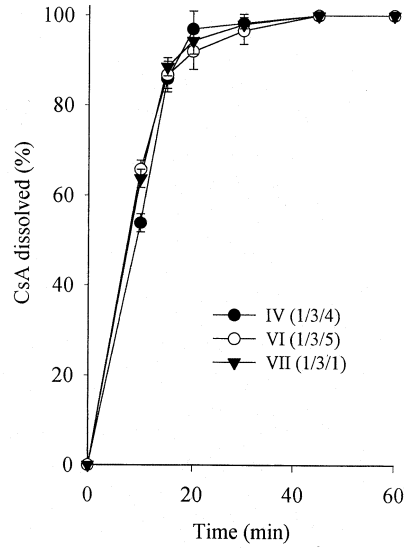

Fig. 4. Effect of dextrin on the dissolution of CsA loaded in CsA–microspheres prepared with different ratio of CsA/SLS/dextrin. Data are expressed as mean ± S.D. ($n = 6$).

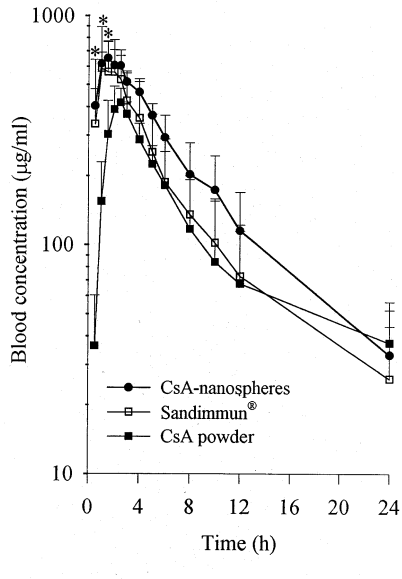

Fig. 5. Plasma concentration profiles of CsA after oral administration of CsA products to dogs. Data are expressed as mean ± S.D. ($n = 4$). (*), $P < 0.05$ by Duncan method of ANOVA when compared to CsA powder.

(Formula VII), and compared with those after oral administration of CsA powder alone and commercial Sandimmun® to dogs, respectively.

Fig. 5 shows the change of whole blood concentration of CsA after oral administration to dogs of CsA products at a dose of 7.6 mg of CsA/kg. The blood concentrations of CsA after a dose of CsA–microspheres were continuously higher than those after the dose of CsA powder alone. This result indicates that CsA–microspheres might be absorbed faster than CsA powder alone in dogs. The blood concentrations of CsA in microspheres were also slightly higher compared to Sandimmun® but there was no statistical significance.

The pharmacokinetic parameters are summarized in Table 2. The T_{max} of CsA–microspheres (1.88 ± 0.48 h) were not significantly different from that from CsA powder alone (2.13 ± 0.48 h). However, CsA–microspheres (0.71 ± 0.08 μg/ml) gave a significantly higher C_{max} of CsA, than did CsA powder alone (0.46 ± 0.15 μg/ml). The AUC of CsA–microspheres (4.85 ± 0.98 h·μg/ml) was 1.7-fold higher than that from CsA powder alone (2.81 ± 1.16 h·μg /ml), indicating that CsA–microspheres improved the bioavailability of CsA. On the other hand, the T_{max} and C_{max} of CsA–microspheres (1.88 ± 0.48 h, 0.71 ± 0.08 μg/ml) were not significantly different from those from Sandimmun® (1.50 ± 0.45 h, 0.72 ± 0.27 μg/ml). The AUC of CsA–microspheres (4.85 ± 0.98 h·μg/ml) was higher than that from Sandimmun® (3.62 ± 1.63 h·μg/ml), but there was no statistical difference between them. Thus, CsA–microspheres gave the bioavailability similar to that of Sandimmun® in dogs.

Table 2
Pharmacokinetic parameters of CsA after oral administration of CsA products to dogs[a]

Parameters	C_{max} (μg/ml)	T_{max} (h)	AUC (h·μ/ml)
CsA powder alone	0.46 ± 0.15	2.13 ± 0.48	2.81 ± 1.16
Sandimmun®	0.72 ± 0.27*	1.50 ± 0.45	3.62 ± 1.63
CsA-microspheres	0.71 ± 0.08*	1.88 ± 0.48	4.85 ± 0.98*

[a] Data are expressed as mean ± S.D. ($n = 4$).
* $P < 0.05$ by Duncan method of ANOVA when compared to CsA powder.

4. Conclusion

The CsA–microspheres prepared with SLS and dextrin gave 1.7-fold higher AUC of CsA compared with CsA powder alone. It is thought to be due to the increase in the dissolution rate of CsA in microspheres. The CsA–microspheres gave a similar AUC to the commercial CsA product, Sandimmun® in dogs. Therefore, dextrin–SLS based CsA–microspheres, showing improved bioavailability of CsA, would be useful to deliver a poorly water-soluble CsA and could be applicable to other poorly water-soluble drugs.

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