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Development of cyclosporin A-loaded hyaluronic microsphere with enhanced oral bioavailability

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Abstract

To develop a hyaluronic microsphere with the improved oral bioavailability of poorly water-soluble cyclosporin A (CsA), the microspheres were prepared with varying ratios of sodium hyaluronate (HA)/sodium lauryl sulfate (SLS)/CsA using a spray-drying technique. The effects of HA and SLS on the dissolution and solubility of CsA in microspheres were investigated. The CsA-microsphere prepared with HA/SLS/CsA at the ratio of 4/2/1 gave the highest solubility and dissolution rate of CsA among those formulae tested. As solubility and dissolution rate of CsA were increased about 17- and 2-fold compared to CsA powder, respectively, this CsA-microsphere was selected as an optimal formula for oral delivery in rats. The CsA-microsphere and Sandimmun neoral sol® gave significantly higher blood levels compared with CsA powder alone. Moreover, the AUC, T_{max} and C_{max} values of CsA in CsA-microsphere were not significantly different from those in Sandimmun neoral sol® in rats, indicating that CsA–microsphere was bioequivalent to the commercial product in rats. Our results demonstrated that the CsA–microsphere prepared with HA and SLS, with improved bioavailability of CsA, might have been useful to deliver a poorly water-soluble CsA.

Keywords: Bioavailability; Cyclosporin A; Sodium hyaluronate; Sodium lauryl sulfate; Microsphere; Spray drying

1. Introduction

Cyclosporin A (CsA) is a lipophilic cyclic undecapeptide of fungal origin, which has the selective property of suppressing various T-lymphocyte functions, particularly the production of interleukin-2 (IL-2) (Christopher et al., 2001). CsA has also been applied in the treatment of patients with selected autoimmune diseases such as rheumatoid arthritis (Sajjadi et al., 1994; Thomson and Neild, 1991). However, it is known that the oral bioavailability of CsA is 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). The conventional formulation of Sandimmun neural sol® is a microemulsion of pre-concentrated CsA designed to provide better consistent absorption of the drug than Sandimmun®. Although this orally administered CsA has more stable drug metabolism, but its gastrointestinal absorption is still incomplete and variable due to its hydrophobic character (Gennery et al., 1999; Tom et al., 2000). Various oral formulations of CsA such as a complexation with cyclodextrin (Miyake et al., 1999) and microsphere (Aberturas et al., 2002; Chacon et al., 1999; Kim et al., 2002a; Urata et al., 1999), nanoparticles (Chacon et al., 1996; Chen et al., 2002; Gref et al., 2001; Guzman et al., 1993; Molpeceres et al., 2000; Ugazio et al., 2002) and microemulsion (Drewe et al., 1992; Gao et al., 1998; Kim et al., 1997; Tejani, 1998) and emulsion (Kim et al., 2002b) have been developed to enhance the solubility, dissolution and bioavailability of CsA. In order to gain more satisfactory release rate, a wider range of

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loading materials is needed to be screened. However, there were still no reports on using sodium hyaluronate (HA) for controlling CsA release. Thus, the development of new oral formulation for the enhancing absorption and bioavailability of systemically effective but poorly absorbed CsA is urgently needed.

Microspheres, in general, have been used as a formulation for improving the solubility, release and bioavailability of poorly water-soluble drugs. Furthermore, spray drying has been commonly used in the pharmaceutical industries for increasing the solubility of poorly water-soluble drugs (Kawashima et al., 1975; Tsuda et al., 1988).

Hyaluronic acid (HA) is a chemically well-characterized linear polysaccharide consisting of alternating b(1 → 4) linked *N*-acetyl-D-glucosamine and b(1 → 3) linked D-glucuronic acid (Jouon et al., 1995; Lapcik et al., 1998). Hyaluronic acid is the only non-sulfated glycosaminoglycan in the extracellular matrix of all higher animals. This polyanionic polymer has a range of naturally-occurring molecular sizes from 1000 to 10,000,000 Da and has unique physicochemical properties and distinctive biological functions (Laurent et al., 1995). Ongoing pharmaceutical and medical research is concentrating on its use in drug delivery systems in addition to its present therapeutic indications in ophthalmology, dermatology and osteoarthritis (Goa and Benfield, 1994; Lapcik et al., 1998). In particular, it was used as carriers in various oral formulations such as microsphere and complex for the improved solubility and bioavailability of poorly water-soluble drugs (Jederstrom et al., 2004; Piao et al., 2007).

Sodium lauryl sulfate (SLS) is an anionic surfactant employed in a wide range of nonparenteral pharmaceutical formulations. SLS was also used as a solubilizer or co-carrier of solid dispersion systems to improve the solubility and dissolution rate of drugs (Ghosh et al., 1998; Khanfar et al., 1997). The CsA-loaded microsphere prepared with SLS and dextrin gave 1.7-fold higher AUC of CsA compared with CsA powder alone (Lee et al., 2001). Thus, in the formulation of CsA-loaded microsphere suitable for solid dosage form, water-soluble HA and SLS were used as a carrier and solubilizer, respectively.

To develop a CsA-loaded microsphere for improving the oral bioavailability, CsA-loaded microspheres were prepared with varying ratios of HA/SLS/CsA using spray-drying technique (Choi and Kim, 2000; Lee et al., 2001). The effects of HA and SLS on the aqueous solubility and dissolution rate of CsA were investigated. The oral bioavailability of CsA-microsphere was then compared with CsA powder alone and commercial Sandimmun neoral sol® in rats.

2. Materials and methods

2.1. Materials

Cyclosporin A was supplied from Hanmi Pharm. Co. Ltd. (Hwaseong, South Korea). Sodium hyaluronate and sodium lauryl sulfate were purchased from Shandong Freda Biochem Co. Ltd. (Jinan, China) and Sigma Chemical Co. (St. Louis, USA), respectively. All other chemicals were of reagent grade and used without further purification.

Table 1
Composition of CsA-loaded microsphere

Ingredients	I	II	III	IV	V	VI	VII
Hyaluronic acid (HA)	4	4	4	4	8	2	0
Sodium lauryl sulfate (SLS)	0	0.5	2	4	0.5	0.5	0.5
Cyclosporin A (CsA)	1	1	1	1	1	1	1

2.2. Preparation of CsA-loaded microsphere

A Buchi 191 nozzle type mini spray-dryer (Model 191, Buchi, Flawil, Switzerland) was used for the preparation of CsA-loaded microsphere. HA, SLS and CsA were dissolved in the mixture of water–ethanol (1:1, v/v). CsA (1 g) and a varying amount of HA, SLS are shown in Table 1. The microspheres were obtained by spray drying through a standard nozzle with an inner diameter of 0.7 mm. The process parameters were set as follows: inlet temperature, 140 °C; outlet temperature, 70–75 °C; and feed flow rate, 4 ml/min; spray air, 3 kg/cm^2 and the flow rate of dry air, about 30 mbar. The direction of air flow was the similar as that of sprayed product (Kim et al., 1994; Lee et al., 1998; Lee et al., 2001).

2.3. Shapes and size of CsA-loaded microsphere

The surface morphology and shape of CsA-loaded microsphere were examined using a scanning electron microscopy (Hitachi S-4100, Tokyo, Japan). The samples were loaded on the specimen stub via double-side sticky tape and coated with gold (Hitachi Iron sputter, E-1030) for 5 min at 100–200 mTorr in a shutter coater before taking photograph at an accelerating voltage of 15 kV. The size of microsphere was also measured using a light scattering spectrophotometer (Nimcomp 370, Particle Sizing System Inc, Santa Barbara, CA, USA).

2.4. Determination of CsA contents in microspheres

For the determination of CsA contents in microspheres, exact amount of microsphere (5 mg) was added to 5 ml of 50% ethanol, shaken in water bath for 3 days and filtered through membrane filter (0.45 μm). The concentration of CsA in the resulting solution was analyzed by HPLC (Jasco PU-987, Japan) equipped with an Inertsil ODS-3 C_{18} column (GL science, 5 μm, 4.6 × 250 mm i.d.), UV detector (Jasco UV-975) and HPLC column temperature controller (Thermasphere™ TS-130, USA). The mobile phase consisted of acetonitrile/water (80:20, v/v) with a flow rate of 1.5 ml/min; the column was thermostated at 70 °C (Francis et al., 2003).

2.5. Solubility of CsA in microsphere

The aqueous solubility study of CsA was measured at 25 ± 1 °C. Excessive amount of each CsA-loaded microsphere or pure CsA was added to 5 ml of water, shaken in water-bath for 3 days. Triplicated samples were centrifuged at 3000 × *g* for 5 min using a centrifuge 5415C (Eppendorf, USA). After being filtered through a membrane filter (0.45 μm), the filtrate

was analyzed using HPLC at 210 nm as described in the above method (Francis et al., 2003).

2.6. In vitro drug release

In order to discriminate the different features in the dissolution rate and apparent solubility of pure CsA and the CsA-loaded microsphere, the dissolution test was performed using USP 26 paddle dissolution apparatus. Pure CsA and CsA-loaded microsphere equivalent to 4.5 mg of CsA were added into 300 ml of distilled water at 37 ± 0.5 °C with a paddle speed of 100 rpm. At predetermined time intervals, 1 ml of the medium was sampled and filtered. The filtrate was analyzed by HPLC at the wavelength of 210 nm as described in the above method (Francis et al., 2003).

2.7. Pharmacokinetics study

2.7.1. In vivo experiments

Male Sprague-Dawley rats weighing 250 ± 20 g were fasted for 24 h prior to the experiments but allowed free access to water. Thirty rats were divided into three groups. The rats in each group were administered with CsA powder alone, Sandimmun neoral sol® and CsA-loaded microsphere [HA/SLS/CsA (4/2/1)] (equivalent to 10 mg/kg of CsA), respectively. All animals care and procedures were conducted according to the Guiding Principles in the Use of Animals in Toxicology, as adopted in 1989 and revised in 1999 by the Society of Toxicology (SOT, 1999).

2.7.2. Administration and blood-collecting

Each rat, anesthetized in an ether-saturated chamber, was secured on a surgical board in the supine position with a thread. A polyethylene tube (PE-50, Intramedic®, Clay Adams, Parsippany, NJ, USA) was inserted into the right femoral artery of the rat, all of the incision was covered with wet cotton. CsA powder and microsphere filled in small hard capsule (#9, Suheung capsule Co. Ltd., Seoul, South Korea), and Sandimmun neoral sol® were orally administered to rats in each group, respectively. Then, 0.4 ml of whole blood samples were withdrawn into tubes with EDTA 3K (1.5 mg/ml) from the femoral artery. They were thoroughly mixed and stored at 4 °C until the assay.

2.7.3. Blood sample analysis

Since 90–98% of CsA is bound to plasma proteins in the blood (Christopher et al., 2001). In this experiment, CsA concentration in whole blood was determined by radiommunoassay (RIA) method using cyclosporin RIA Kit (Immunotech Inc., Westbrook ME, USA). Aliquots of 20 μl of blood sample were transferred into polystyrene tubes and 20 μl of control and standard, after adding 500 μl of ^{125}I-Cyclosporin, and incubated for 1 h at 20–25 °C. After centrifugation and decanting of the supernatant, the bound radioactivity of calibrators, controls and samples were measured. All calibrators, controls and smaples were analysed in duplicate. The radioactivity was measured using automatic gamma counter (1470 wizard γ-counter, Turku, Finland). The calibration range of RIA method was 25–2000 ng/ml, with a functional sensitivity calculated at about 25 ng/ml.

2.7.4. Pharmacokinetic data analysis

Pharmacokinetic parameters associated with each group were estimated by non-compartmental method using WINNONLIN (Version 1.1, Scientific Consulting Inc., NC, USA; Gabrielsson and Weiner, 1999). The maximum whole blood concentration of drug (C_{max}) and time to reach maximum whole blood concentration (T_{max}), half-life ($t_{1/2}$) and area under the drug concentration–time curve (AUC) were obtained from whole blood data. All results were expressed as mean ± standard deviation.

3. Result and discussion

3.1. Physicochemical properties of CsA-loaded microspheres

CsA-loaded microsphere was prepared with various ratios of HA/SLS/CsA using a spray-dryer. The compositions of the

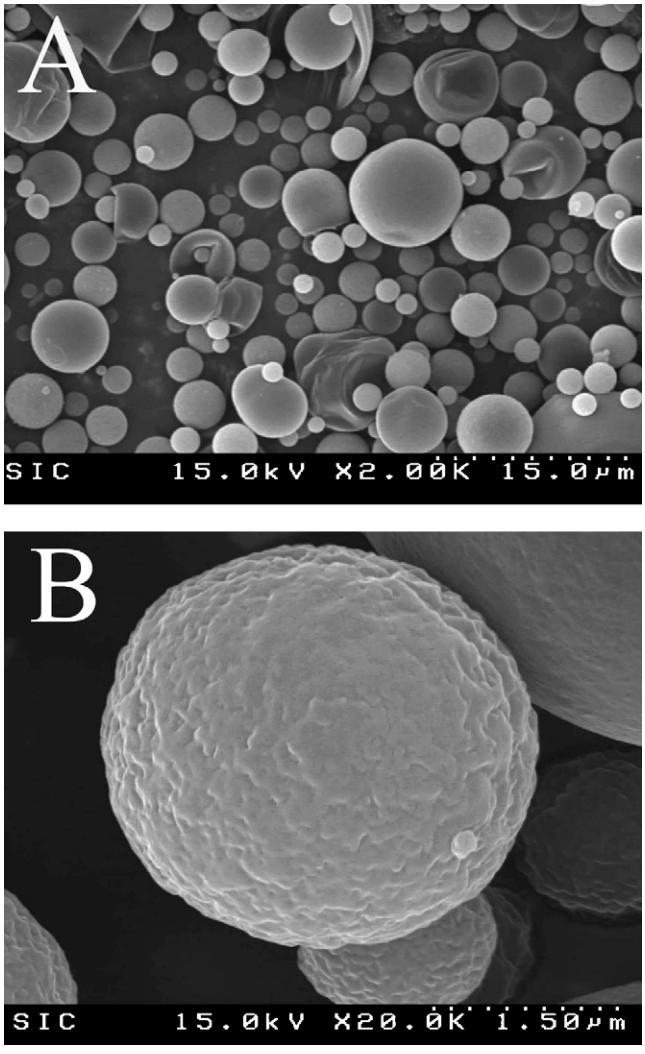

Fig. 1. Representative scanning electron micrographs of CsA-loaded microsphere: (A), (×2000); (B), (×20,000).

CsA-loaded microsphere are illustrated in Table 1. In preliminary experiment, the change in the contents of CsA was not significantly observed, after only CsA dissolved in the mixture of water–ethanol (1:1, v/v) was spray-dried at the inlet temperature of 140 °C and outlet temperature of 70–75 °C. Thus, CsA was thermally stable in the preparation of CsA-loaded microsphere. The CsA-loaded solutions were thought to be instantaneously spray-dried at the relatively higher inlet temperature and instantly moved in the collecting chamber at room temperature.

The scanning electron micrograph of the CsA-loaded microsphere showed that the major particles were spherical in shape with a smooth surface as shown in Fig. 1. The geometric mean diameters of the CsA-loaded microsphere were about 2.5 μm. Furthermore, the various ratio of HA/SLS/CsA did not significantly affect their particle sizes (Fig. 2).

In this study, the weight of CsA theoretically contained in the microsphere was compared with the weight actually obtained from the drug content studies, i.e., the quantity loaded into the microsphere formulated, to get the CsA-loading efficiency (Attama and Mpamaugo, 2006). Following equation was used for the calculation.

$$\text{drug-loading efficiency}\,(\%) = \left(\frac{C_p}{C_t}\right) \times 100 \quad (1)$$

where C_p and C_t were the actual and theoretical drug content in CsA-loaded microsphere, respectively.

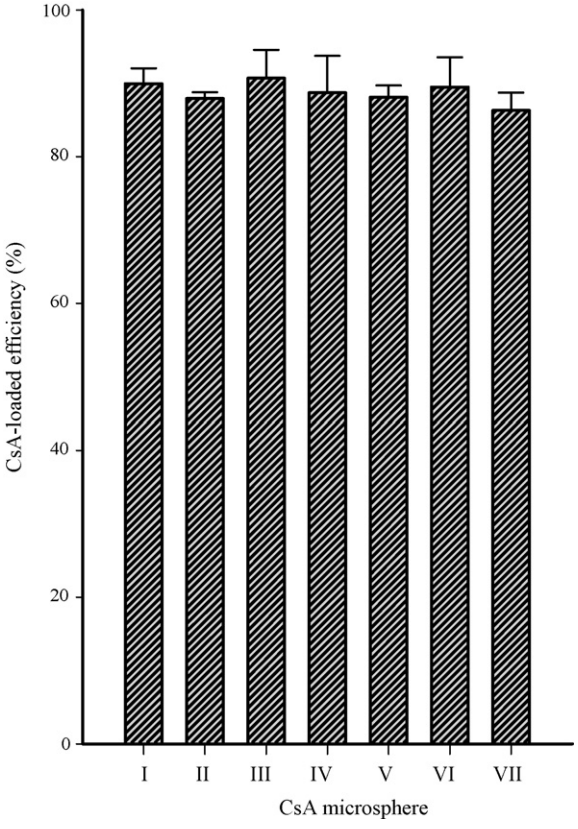

Fig. 2. Particle size of CsA-loaded microsphere. CsA-loaded microsphere was composed of various ratios of HA/SLS/CsA. Each value represents the mean ± S.D. ($n = 3$).

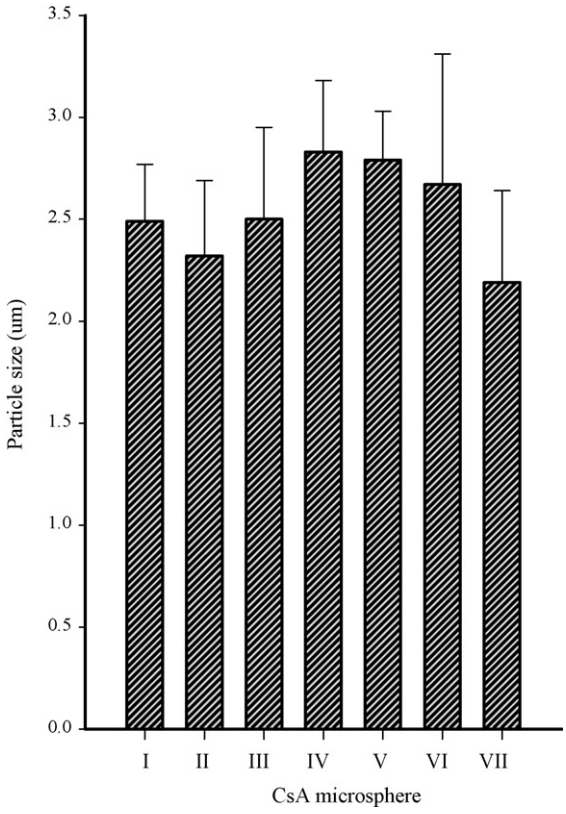

Fig. 3. Drug-loading efficiency of CsA-loaded microsphere. CsA-loaded microsphere was composed of various ratios of HA/SLS/CsA (0–8/0.5/1). Each value represents the mean ± S.D. ($n = 3$).

The drug-loading efficiency of CsA-loaded microsphere was about 85–90% in all the formulations and the drug-loading efficiency did not show significant difference (Fig. 3).

The HA markedly increased the solubility of CsA in CsA-loaded microsphere (Fig. 4). Furthermore, the solubility of CsA in CsA-loaded microsphere abruptly increased as the amount of SLS increased to 2 g (Fig. 5). It indicated that the solubilization of CsA was greatly affected by the surfactant (Lee et al., 2001). Moreover, compared to their particle size and drug-loading efficiency, there was no correlation between their solubility value and them (Figs. 2–3 and Fig. 5).

3.2. In vitro drug release

To evaluate whether HA affected the dissolution rates of CsA from CsA-loaded microsphere, we performed the dissolution test on four formulae of CsA-loaded microspheres (Table 1, formula II and V–VII) compared with CsA powder. The dissolution profiles of CsA from them are illustrated in Fig. 6. The dissolution rates of drug from four microspheres (formula II and V–VII) significantly increased compared to CsA powder. Among three microspheres with HA (formula II, V and VI) tested, microsphere with 8 g HA (formula V) gave the highest dissolution rates of CsA. Furthermore, the dissolution rate of CsA increased with increasing the content of HA. However, the formula VII without HA showed significantly lower dissolution rate than another microspheres. Our results suggested that the

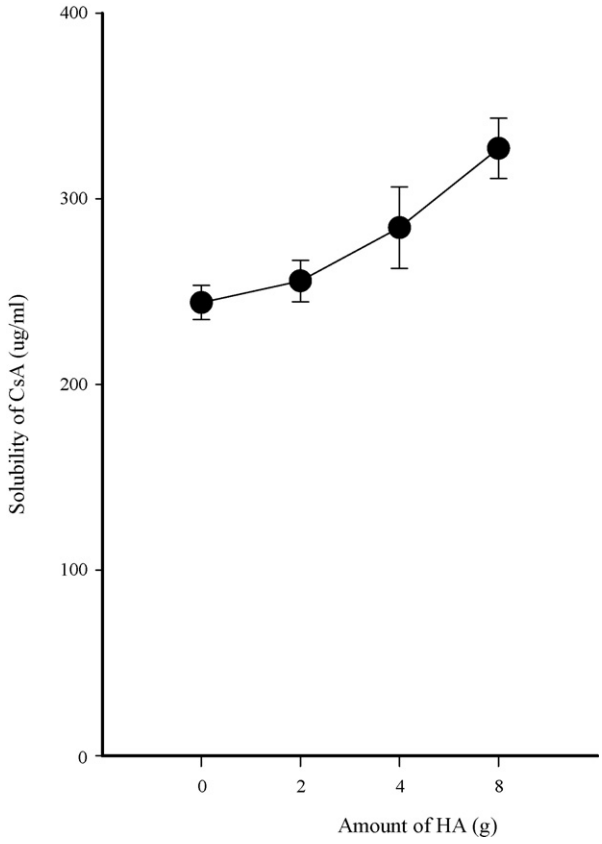

Fig. 4. Effect of HA on the solubility of CsA in CsA-loaded microsphere. CsA-loaded microsphere was composed of various ratios of HA/SLS/CsA. Each value represents the mean ± S.D. ($n = 3$).

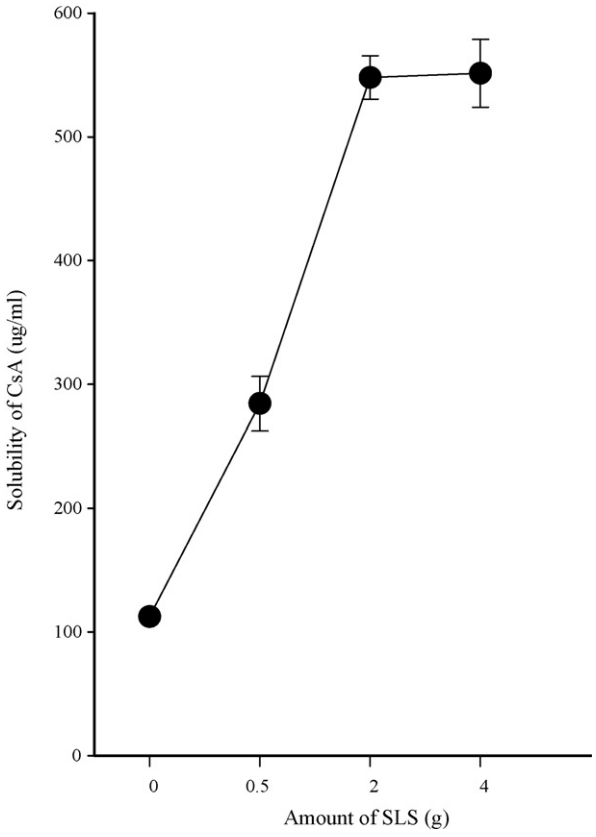

Fig. 5. Effect of SLS on the solubility of CsA in CsA-loaded microsphere. CsA-loaded microsphere was composed of various ratios of HA/SLS/CsA (4/0–0.5/1). Each value represents the mean ± S.D. ($n = 3$).

HA in CsA-loaded microsphere could improve the dissolution rate of CsA in water because the hydrophilic polymers followed by spray-drying result in a drug powder with a markedly enhanced drug dissolution rate. A high and hydrophilized surface area is created (Rasenack and Muller, 2002). HA was very easily dissolved in water, because a number of hydrogen bonds were present between water molecules and HA (Kaufmann et al., 1998).

To evaluate whether SLS affected the dissolution rates of CsA from CsA-loaded microsphere, we performed the dissolution studies on three formulae of CsA-loaded microsphere (Table 1, formula I, II, III and IV) compared with CsA powder. The dissolution profiles of CsA from them are illustrated in Fig. 7. The dissolution rates of drug from all CsA-loaded microsphere significantly increased compared to CsA powder alone. Moreover, the microsphere showed the dissolution rate of drug increased with increasing the content of SLS, but there were no significant differences between microspheres formula III and IV. Our results suggested that the SLS was useful for improving the dissolution rate of poorly water-soluble CsA in the microsphere (Lee et al., 2001). SLS was used as a solubilizer or co-carrier to improve the dissolution rate of drugs (Khanfar et al., 1997; Ghosh et al., 1998).

On the other hand, the dissolution rate of CsA prepared with SLS alone (formula VI) and HA alone (formula I) was significantly higher than that of CsA powder alone, indicating that the SLS-based solid dispersion system and HA-based solid dispersion also improved the dissolution rate of CsA. The CsA-loaded microsphere (formula II, III, IV, V and VI) with HA and SLS further improved the dissolution rates of CsA. Formula III, IV and V showed similar dissolution rate of CsA. The formula III and IV gave higher solubility of CsA than formula V. Moreover, formula III gave higher drug content of CsA than formula IV (Table 2).

In conclusion, the amount of drug dissolved from the CsA-loaded microsphere, composed of [HA/SLS/CsA (4/2/1)] (formula III), in water for 4 h increased about 2-fold compared to CsA powder (88.46 ± 1.32% vs. 43.42 ± 7.86%) and gave the highest solubility (about 500 μg/ml) of CsA. Thus, this composition was selected as an optimal formula

Table 2
Pharmacokinetic parameters of CsA delivered by CsA powder, Sandimmun neoral sol® and CsA-loaded microsphere

Parameters	Pure CsA	CsA-loaded microsphere	Sandimmun neoral sol®
T_{max} (h)	4.00 ± 2.29	3.21 ± 1.43	3.00 ± 0.00
C_{max} (μg/ml)	0.95 ± 0.43	1.90 ± 0.21*	1.72 ± 0.20*
AUC (h μg/ml)	10.43 ± 4.21	21.93 ± 5.62*	20.41 ± 5.11*
$t_{1/2}$ (h)	4.35 ± 1.05	6.33 ± 2.00	6.85 ± 2.11
K_{el}	0.16 ± 0.08	0.11 ± 0.05	0.10 ± 0.05

Data were expressed as the mean ± S.D. ($n = 10$).
* $P < 0.05$ compared with pure CsA.

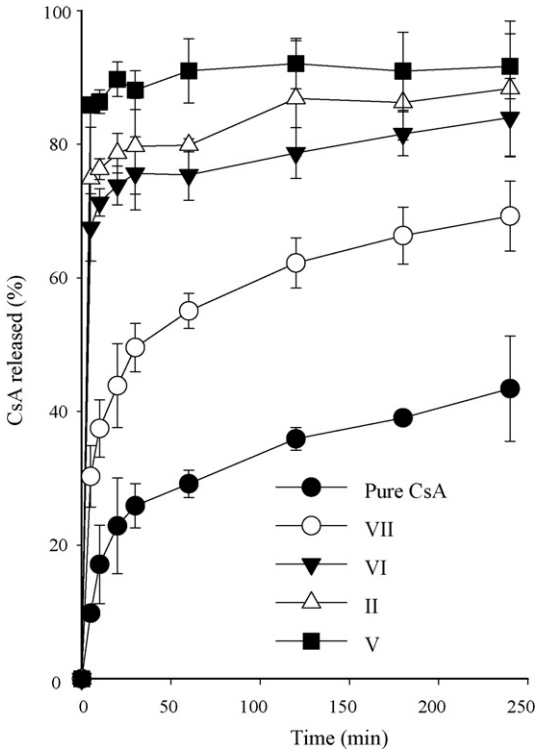

Fig. 6. Effect of HA on the release of drug from CsA-loaded microsphere. CsA-loaded microspheres VII, VI, II and V were composed of various ratios of HA/SLS/CsA (0/0.5/1), (2/0.5/1), (4/0.5/1) and (8/0.5/1), respectively. Each value represents the mean ± S.D. ($n = 3$).

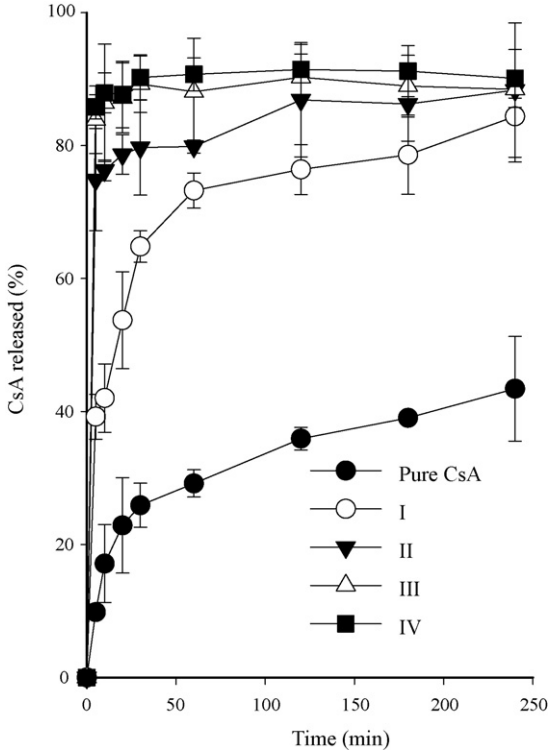

Fig. 7. Effect of SLS on the release of drug from CsA-loaded microsphere. CsA-loaded microspheres I, II, III and IV were composed of various ratios of HA/SLS/CsA (4/0/1), (4/0.5/1), (4/2/1) and (4/3/1), respectively. Each value represents the mean ± S.D. ($n = 6$).

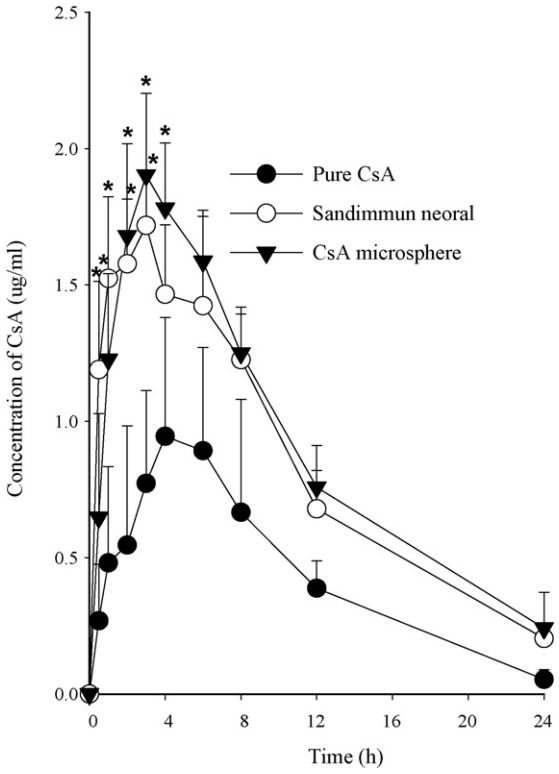

Fig. 8. Whole blood concentration–time profiles of CsA after oral administration of CsA powder, Sandimmun neoral sol® and CsA-loaded microsphere to rats. CsA-loaded microsphere was composed of HA/SLS/CsA (4/2/1). Each value represents the mean ± S.D. ($n = 10$). *$P < 0.05$ compared with pure CsA.

of CsA for the oral delivery system in the pharmacokinetics study.

3.3. Pharmacokinetics study

The pharmacokinetic parameters of CsA were determined after oral administration of CsA powder, Sandimmun neoral sol® and CsA-loaded microsphere (formula III). Fig. 8 shows the change of mean whole blood concentration of CsA after oral administration of preparations in rats. The initial plasma concentrations of CsA in the microsphere were significantly higher compared with those in CsA powder ($P < 0.05$). Furthermore, CsA-loaded microsphere gave relatively higher initial plasma concentrations of CsA than did Sandimmun neoral sol® to 6 h, but there were no significant differences. Our results suggested that the higher initial plasma concentrations of CsA in the microsphere were due to the increase in dissolution rate of CsA in the microsphere.

The pharmacokinetic parameters are shown in Table 2. The C_{max} and AUC of CsA in CsA powder, Sandimmun neoral sol® and microsphere were 0.95 ± 0.43 μg/ml and 10.43 ± 4.21 h μg/ml; 1.72 ± 0.20 μg/ml and 20.41 ± 5.11 h μg/ml; 1.90 ± 0.21 μg/ml and 21.93 ± 5.62 h μg/ml, respectively. The microsphere and Sandimmun neoral sol® gave significantly higher blood levels compared with CsA powder alone. However, the AUC, T_{max} and C_{max} values of CsA in the microsphere were not significantly different from those in

Sandimmun neoral sol® in rats, indicating that CsA-microsphere was bioequivalent to Sandimmun neoral sol® in rats. Furthermore, our results suggested that the enhanced oral relative bioavailability of CsA in the microsphere was contributed by the increase in initial dissolution rate of CsA from the microsphere in gastrointestinal tract transit time (Gao et al., 1998) and intimacy of the drug with the absorbing membrane brought about by the bioadhesive property of HA (Huang et al., 2007; Jederstrom et al., 2005). Thus, the CsA-loaded microsphere prepared with HA and SLS could improve the oral bioavailability of CsA.

4. Conclusion

The CsA-loaded microsphere developed using spray-drying technique with HA and SLS gave 2-fold higher dissolution rate and 2-fold higher AUC of CsA compared with CsA powder and bioequivalent to Sandimmun neoral sol® in rats, indicating that the more drug from CsA-loaded microsphere could be orally absorbed in rats, leading to a more effective oral dosage form for poorly water-soluble CsA. A further study on the oral bioavailability in human subjects of CsA-loaded preparation will be performed.

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