Enteric Solid Dispersion of Cyclosporin A (CyA) Having Potential to Improve Availability of CyA in Rabbit

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The availability of cyclosporin A (CyA) administered as an enteric solid dispersion preparation of which the composition is CyA : HCO-60® : HP-55® = 1 : 2 : 8 was evaluated in rabbits. The additives are surfactant (polyoxyethylated, 60 μmol, castor oil derivative, HCO-60®) and enteric coating material (hydroxypropylmethyl cellulose phthalate, HP-55®), which are generally used as pharmaceutical additives. Both the systemic and lymphatic availabilities of CyA from this solid preparation were measured in rabbits after intrastomach administration, 7 mg CyA/kg, and were compared with those from conventional oily solution, Sandimmun®. The mean systemic availability of CyA from the solid preparation was 57% which is about 1.5 times greater than that obtained from Sandimmun. The amounts of CyA transferred into the thoracic lymphatics within 12 h from solid dosage form and Sandimmun are 0.62 ± 0.16(S.D.)% and 0.13 ± 0.05% of the administered CyA dose. These results support the usefulness of the new solid dosage form of CyA.

Keywords cyclosporin A; oral dosage form; enteric solid dispersion; systemic availability; lymphatic availability; plasma level; lymph level; rabbit; bioavailability

Introduction

Cyclosporin A (CyA) has a strong immunosuppressive effect and is used extensively in the field of organ transplantation.[1,2] Based on the assumption that the immunosuppressive effect of CyA is related to its concentration in the lymphatic system where lymphocytes exist in large quantities, we developed a new enteric solid dispersion system of CyA.[3-9] The *in vitro* dissolution behavior of CyA from this new oral solid dosage form, and both the systemic and lymphatic availabilities of CyA from this dosage form were studied in rats.[10] Both availabilities were dependent on the quality of the enteric materials such as cellulose acetate phthalate (CAP), methacrylic acid and methacrylic acid methyl ester copolymer (Eudragit L-100®) and hydroxypropylmethylcellulose phthalate (HP-55®), and the quantity of the surfactants, polyoxyethylated, 60 μmol, castor oil derivative (HCO-60®), which is generally used as a pharmaceutical additive. The HP-55 preparation having the composition CyA : HCO-60 : HP-55 = 1 : 2 : 8 showed the highest systemic and lymphatic availabilities of CyA. Finally, this new dosage form must be evaluated clinically using tansplant patients. However, before performing this final study, an animal scale-up study must be performed. As a first approach to this animal scale-up process, an availability study was performed with rabbits and this report presents the results.

Experimental

Materials CyA and CyD (cyclosporin D, used as an internal standard for high performance liquid chromatography (HPLC) analysis) powders were kindly supplied by Sandoz Ltd., Basle, Switzerland. Sandimmun® (CyA concentration 100 mg/ml) was obtained from Japan Sandoz Ltd. (Tokyo, Japan). HCO-60® was obtained from Nikko Chemicals Co., Ltd. (Tokyo, Japan). HP-55® was obtained from Shin-Etsu Chemical Industry, Co., Ltd. (Tokyo, Japan). All other reagents were commercial products of reagent grade.

Preparation of Enteric Solid Dispersion of CyA The chemical composition of the enteric solid dispersion system of CyA is CyA : HCO-60 : HP-55 = 1 : 2 : 8. These three components were first dissolved in methanol at 40 °C. The resultant solution was transferred into a mortar. Stirring was continued at room temperature (25 °C) until the smell of the solvent disappeared. The resultant granules were dried under vacuum overnight at room temperature. After being pulverized in a mortar with a pestle, the dried granules were screened through a 50 mesh screen to obtain fine granules. After measuring the body weight of the experimental animals, the calculated amount of thus obtained CyA fine granules was packed into a gelatin capsule (JP XI, #1 capsule).

Availability Study Four white albino rabbits, weighing 3 to 4 kg, were used for each CyA preparation. The rabbits were fasted overnight but had free access to water. Under anesthesia by intravenous (i.v.) injection of sodium pentobarbital, 45 mg/kg, a polyethylene cannula (i.d., 0.5 mm; o.d., 0.8 mm; Dural Plastics, Australia) was surgically introduced into the left carotid artery to obtain blood samples. A modification of the method of Ellis[11] was used for the collection of lymph from the venous angle. A heparin-filled polyvinyl cannula (i.d., 0.5 mm; o.d., 1.2 mm; Dural Plastics) was threaded about 3 mm into the thoracic lymph duct. A drop of tissue cement (Aron Alpha®, Sankyo Co., Tokyo) was applied to the hole in the lymphatic vessel to seal it and to fix the cannula in place. After collecting blank blood and lymph samples, CyA preparations were administered into the stomach of the rabbit. In the case of CyA granules (*i.e.*, enteric solid dispersion), a capsule into which fine CyA granules had been packed was inserted into the rabbit stomach through an incision, 0.5 mm. The administered amount of CyA granules was 77 mg/kg of rabbit body weight, corresponding to a CyA dose of 7 mg/kg. After administration, the gastric incision was sutured and sealed with tissue cement. The output of lymph from the thoracic lymph duct was collected in hourly fractions in tared culture tubes for 12 h, and the volumes determined gravimetrically. Single blood sample (100—200 μl) was also obtained at 1, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 7, 8, 9, 10 and 12 h after administration. All the blood samples were immediately centrifuged at 37 °C to obtain the plasma fraction. In the case of the administration of Sandimmun, a 70 μl alliquot of the oily solution, 100 mg/ml, per kg of rabbit body weight was directly injected into the rabbit stomach with a microsyringe and the pore made was fixed with a drop of tissue cement. Between samplings, the cannula was filled with heparinized saline. For the i.v. study, CyA solution, 21—28 mg/ml, prepared with saline containing 1 w/v% HCO-60 was infused into the rabbit ear vein for 1 h at the rate of 1 ml/h using a compact infusion pump (Model KN-201, Natsume Co., Ltd., Tokyo) at a dose level of 7 mg/kg. The blood samples were collected throughout the infusion period at 0, 20, 40, 60 and up to 480 min. The plasma was immediately obtained by centrifugation. All the plasma and lymph samples were immediately frozen in a deep freeze at −20 °C until analyzed.

Drug Assay The analytical method used was basically similar to the HPLC assay method developed in our laboratory.[12] After defrosting of the plasma or lymph samples, 100 to 200 μl aliquots of the samples were used for the CyA assay after extraction into diethyl ether. The extraction procedure was the one we reported before.[12] Briefly, after washing the residue of the ether extract with hexane, CyA was reextracted into a mixture of carbon tetrachloride and 0.5 N NaOH (5 : 1). The separated carbon tetrachloride phase was transferred to a clean tube and evaporated to dryness with a stream of nitrogen at 50 °C. The resulting residue was dissolved in 200 μl of the mobile phase. An aliquot of 150 μl was then injected onto the column. A Hitachi 655 pump (Tokyo, Japan) and a Rheodyne 7125 sample injector were used for chromatographic analysis. The analytical column was a RP-18 Chemcosorb 5 μm (25 × 0.46 cm, Chemco Scientific Co., Ltd., Osaka, Japan). The UV detector was a

Hitachi 638-41. The column was maintained at 75 °C with a column heater. The mobile phase was composed of acetonitrile–water (70 : 30), and the flow rate was 1 ml/min (60 kg/cm^2). CyA and CyD (used as an internal standard) were detected at 205 nm. Under these conditions, the retention times were 7 min for CyA and 9.5 min for CyD. No interfering peak was detected in the plasma or lymph samples used as blanks or in those from rabbits given CyA. The concentration of CyA in the biological fluids was determined from calibration curves of peak area ratios of CyA to CyD. The standard curves of CyA added to rabbit plasma and lymph samples were linear over the range of 0.1—20 μg/ml and passed through the origin.

Estimation of Lymphatic Availability The lymphatic availability of CyA was estimated as the percentage amount of CyA transferred to the thoracic lymphatics up to the end of the experiment, 12 h after the oral administration of CyA preparations to rabbits. As our previous report suggested that the amount of CyA transferred from the systemic circulation into the thoracic lymphatics is negligible,[9] the percentage recovery of CyA in the thoracic lymphatics may be a reasonable index for lymphatic availability of CyA.

Analysis of the Data The terminal elimination rate constant, β, for the CyA concentration–time curves after i.v. or oral administration was determined by linear regression based on at least 4 data points from the terminal portion of the plasma concentration–time plots. The areas under the plasma concentration–time curve after administration, $AUC_{i.v.}$ and AUC_{oral}, were calculated to maximum concentration with the linear trapezoidal rule and after that to the last measured plasma concentration, $C_{p(last)}$, with the logarithmic trapezoidal rule with the addition of a correction term after the last measured point to infinity, namely $C_{p(last)}/\beta$. The values are expressed as their mean ± S.D. unless otherwise noted. Statistical differences were assumed to be reproducible when $p<0.05$ (two-sided t-test).

Results

The rabbit plasma CyA concentration *vs.* time curve after administration of an enteric solid dispersion preparation of CyA at the CyA dose level of 7 mg/kg is represented in Fig. 1 along with that obtained after oral administration of Sandimmun, at the same dose of CyA. For Sandimmun, the peak CyA level, 0.53 ± 0.11 μg/ml, appeared at 3.5 h after administration. In the case of the enteric solid dispersion preparation, a long absorption lag-time, about 1.5 h, was observed, and the peak plasma CyA level appeared at 5 h (0.74 ± 0.11 μg/ml). Figure 1 also shows the mean plasma concentration–time curve for CyA over the entire period of the i.v. infusion study for the four rabbits in whom a constant rate infusion of CyA was performed for 1 h at a CyA dose level of 7 mg/kg. The plasma CyA levels gradually increased until the end of the infusion, and after that declined rapidly. The mean peak level was 2.96 ± 0.33 μg/ml. The parameters concerning the systemic availability of CyA are summarized in Table I. The calculated AUC_{oral} values are 2.11 ± 0.33 μg·h/ml for Sandimmun and 3.02 ± 0.06 μg·h/ml for the enteric solid dispersion preparation. In addition, the mean $AUC_{i.v.}$ value obtained after i.v. infusion of CyA is 5.30 ± 0.18 μg·h/ml. By comparing the AUC values for oral preparations, AUC_{oral}, to that for i.v. preparation, $AUC_{i.v.}$, we may estimate the oral availabilities (F) of CyA from the two oral preparations. Namely, F = 0.40 for Sandimmun and F = 0.57 for enteric solid dispersion preparation. In addition, the relative availability of CyA from enteric solid dispersion preparation against Sandimmun was estimated to be 1.43. This implies that the new enteric solid dispersion preparation shows about 1.5 times greater extent of bioavailability (EBA) than the conventional olive oil CyA preparation.

The lymphatic CyA concentration *vs.* time curves for the two oral preparations are shown in Fig. 2, and Table II gives the lymphatic availability of CyA from the two dosage forms. The peak lymph CyA level, 4.60 ± 1.32 μg/ml, appeared in the 2—3 h sample for Sandimmun. However, the peak lymph CyA level, 21.23 ± 8.28 μg/ml, was observed at 5—6 h for enteric solid dispersion preparation. As the lymph flow did not show a significant difference between the two oral preparations, 1.47 ± 1.32 ml/h for Sandimmun and 1.54 ± 0.40 ml/h for enteric solid dispersion preparation, the amount of CyA transferred into the thoracic lymphatics was also dependent on the preparations. Namely, the

Table I. Systemic Availability of CyA in Rabbits

Preparation	AUC[a] (μg·h/ml)	Bioavailability, F (%)
IV preparation	5.30 ± 0.18	100
Oral preparation		
Enteric solid dispersion	3.02 ± 0.06	57
Sandimmun	2.11 ± 0.33	40

a) Each value represents the mean ± S.E.

Table II. Lymphatic Delivery of CyA in Rabbits

Preparation	Peak lymph CyA level (μg/ml)	Percentage of CyA transferred over experimental period[a] (% of dose)	Lymph flow (ml/h)
Enteric solid dispersion	21.23 ± 8.28[b]	0.62 ± 0.16[b]	1.54 ± 0.40
Sandimmun	4.60 ± 1.32	0.13 ± 0.05	1.47 ± 1.32

a) Lymph was collected from the venous angle over 12 h. *b*) A statistically significant difference from the value of Sandimmun by Student's t test ($p<0.05$). Each value represents the mean ± S.E.

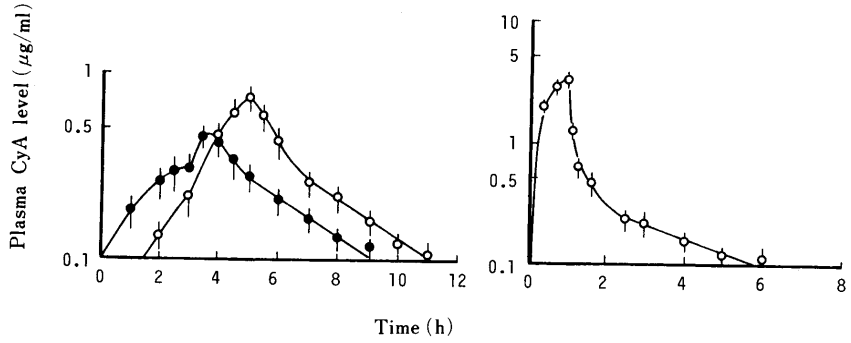

Fig. 1. Plasma Concentration–Time Curves of CyA after Oral Administration of Two CyA Preparations, Enteric Solid Dispersion System (○) and Sandimmun (●), (Left), and i.v. Preparation (Right) at the Dose Level of 7 mg of CyA/kg of Rabbit Body Weight

Each point is the mean of four individual determinations and is expressed as the mean ± S.E.

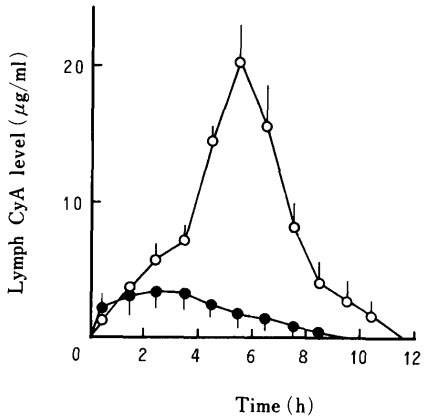

Fig. 2. Lymphatic Concentration of CyA after Oral Administration of Two Preparations, Enteric Solid Dispersion System (○) and Sandimmun (●), at the CyA Dose Level of 7 mg/kg

Each point is the mean of four individual determinations and is expressed as the mean ± S.E.

percentages of the administered CyA dose transferred into the thoracic lymphatics in 12 h were 0.62±0.16% for enteric solid dispersion preparation and 0.13±0.05% for Sandimmun.

Discussion

CyA is an extremely valuable immunosuppressant in transplantation immunology, increasing the survival of transplants by inhibiting the T lymphocyte compartment responsible for rejection.[13-16] However, CyA also exhibits a number of side effects such as nephrocytotoxicity.[17] In addition, the bioavailability of CyA in renal tansplant patients is reported to range from almost zero to 40% after oral administration of CyA as an conventional olive oil preparation.[18,19] As CyA is insoluble in water, olive oil solution was used at the initial stage of the pharmacological studies.[20] This preparation continued to be used in the consequent studies. However, such a primitive preparation has many problems. For example, the oily flavor is unpalatable to patients. In addition, the compliance of the patient is difficult to ensure. To overcome such problems, a soft gelatin capsule containing CyA dissolved in olive oil has been developed recently and bioequivalence was confirmed by a clinical pharmacological study using renal transplant patients.[21] This preparation overcomes the above problems. However, the main pharmaceutical additive is olive oil. On the other hand, our new enteric solid dispersion preparation can also solve these problems. Furthermore, greater systemic and lymphatic availabilities are obtained from our new preparation as compared to those obtained from Sandimmun. As the lymphatic availability of CyA is related to its pharmacological action,[7] our enteric solid dispersion preparation is thought to be superior to the CyA soft gelatin capsule preparation.

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