Enteric Solid Dispersion of Ciclosporin A (CiA) Having Potential to Deliver CiA into Lymphatics

Kanji TAKADA,* Mamoru OH-HASHI, Yoshihiro FURUYA, Hiroshi YOSHIKAWA and Shozo MURANISHI

Department of Biopharmaceutics, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607, Japan. *Received August 5, 1988*

Solid dispersions composed of three components, ciclosporin A (CiA), surfactant (HCO-60) and a pharmaceutical additive, were prepared. As an additive, cellulose acetate phthalate (CAP), methacrylic acid and methacrylic acid methylester copolymer (Eudragit L-100®) and hydroxypropylmethylcellulose phthalate (HP-55®), which are generally used as enteric coating materials, were employed. The dissolution behavior of CiA from these enteric solid-dispersion system was studied according to the paddle method of JP XI in comparison with that of Sandimmun®, an olive oily CiA solution as a reference. Solid dispersion of CiA preparation did not dissolve in the 1st test fluid (pH 1.2) in 2 h. In the 2nd fluid (pH 6.8), about 80% of CiA was dissolved within 12 min, though the dissolution rate was dependent on both the quality and quantity of the additives. An *in vivo* systemic and lymphatic availability study was performed with rats whose carotid artery and thoracic lymph duct were cannulated. After intrastomach administration of each CiA preparation to rats at a dose of 7 mg/kg, blood and lymph samples were collected for 6 h. One of the HP-55 preparations gave the highest plasma CiA level, $C_{max} = 0.99 \pm 0.20$ (S.E., $n = 4$) μg/ml, and also showed the highest lymphatic availability, the percentage of dose delivered to the lymphatics in 6 h was 1.98 ± 0.10% and the maximum lymph CiA level was 76.8 ± 12.86 μg/ml. Lymphatic availability of CiA from Sandimmun was 0.78 ± 0.11% and the peak plasma CiA level was 0.46 ± 0.10 μg/ml. These results support the usefulness of the new enteric solid dispersion system for oral delivery of CiA.

Keywords ciclosporin A; dosage form; enteric solid dispersion; systemic availability; lymphatic availability; plasma level; lymph level; rat

Introduction

Ciclosporin A (CiA) is a cyclic undecapeptide produced as a metabolite in submerged cultures of the soil fungus *Tolypocladium inflatum* GRAMS.[1] CiA is clinically used as an immunosuppressant in the field of organ transplantation.[2] CiA appears to act on the immune system by inhibiting the initial step of T-lymphocyte activation,[3,4] and diminishes the responsiveness of helper-inducer T-lymphocytes to interleukin-1. CiA also inhibits the production of interleukin-2 by alloantigen- and lectin-stimulated T-lymphocytes and prevents the expression of receptors for interleukin-2 by precursor cytolytic T-lymphocytes.[5,6] Therefore, the immunosuppressive activity of CiA is related to a selective action against T-lymphocytes. The lymphocytes, including T-lymphocytes, circulate mainly in the lymphatic systems in the body. Therefore, the immunosuppressive activity of CiA may be related to the CiA concentration in the lymphatic system. Based on this assumption, we developed a new CiA carrier, HCO-60 (polyoxyethylated, 60 μmol, hydrogenated caster oil), with selective lymphatic transporting ability.[7-10] The lymphatic CiA levels from our new well-solubilized formulation with HCO-60 were about twenty times greater than from olive oil formulation.[8] A basic pharmacological study using the rat heart transplantation model showed that the mean survival time (in days) of the transplanted rat heart was significantly longer with our new formulation than with the usual olive oil formulation.[11] Moreover, we studied the pharmacokinetics of orally administered CiA and some pharmaceutical and biological factors affecting the systemic and lymphatic availabilities of CiA, and learned that greater systemic and lymphatic availabilities were obtained after the intraduodenal administration of CiA solution than after administration into the stomach.[12,13] Therefore, an enteric dosage form would be desirable for oral CiA therapy with our new delivery system. However, the precise studies were performed with a CiA solution, which is not very convenient. Therefore, we tried to develop a solid CiA dosage form which would retain the potential to deliver more CiA to the lymphatics after being absorbed from the gastrointestinal tract.

Experimental

Materials CiA and ciclosporin D (CiD; used as an internal standard) powders were kindly supplied by Sandoz Ltd., Basle, Switzerland. Sandimmun® (CiA concentration 100 mg/ml) was obtained from Japan Sandoz Ltd. (Tokyo, Japan). HCO-60 was obtained from Nikko Chemicals Co., Ltd. (Tokyo, Japan). Cellulose acetate phthalate (CAP) was obtained from Wako Pure Chemicals Co., Ltd. Methacrylic acid and methacrylic acid methyl ester copolymer (Eudragit L100® made by Rohm Pharm. Co., Ltd., Darmstadt, W. Germany) and hydroxypropyl methylcellulose phthalate (HP-55®) were obtained from Higuchi Trading company (Tokyo, Japan) and Shin-Etsu Chemical Industry Co., Ltd. (Tokyo, Japan), respectively. All other reagents were commercial products of reagent grade.

Preparation of Pulverized CiA Preparations. The chemical composition of each sample is shown in Table I. CiA, HCO-60 and an enteric pharmaceutical additive were dissolved in 10 ml of 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.

Dissolution Study The dissolution study of CiA from the individual test preparation was performed according to the procedure of the paddle method (JP XI). The dissolution media employed were JP 1st fluid (JP XI, pH 1.2) and 2nd fluid (JP XI, pH 6.8). A sample of fine granules

TABLE I. Composition of Enteric Pulverized CiA Preparations

Preparation	Amount of CiA (mg)	Amount of HCO-60 (mg)	Amount of enteric additive (mg)
CAP	100	250	800
Eudragit	100	150	250
HP-55 #1	100	250	750
HP-55 #2	100	200	800
HP-55 #3	100	100	800
HP-55 #4	100	50	850

containing 10 mg of CiA was put into a bag made of tissue paper (1 × 1 cm). In the case of Sandimmun, a 100 μl aliquot of the oral oily solution was also put into a tissue paper bag and used for dissolution study. After being enclosed in the sinker, the paper bag was put into 500 ml of JP 1st fluid maintained at 37 °C. The shaft was then placed in the fluid at 37 °C and the paddle was rotated at 150 rpm. Aliquots (100 μl) of the sample were taken at 0, 1, 2, 4, 6, 8, 10, 12, 14, 16, 20, 30, 60 and 120 min. For enteric preparations, the test medium was changed to JP 2nd fluid at 120 min and the dissolution study was performed for more than 2 h. Aliquots (100 μl) of the sample were also taken at 120, 121, 122, 124, 126, 128, 130, 132, 134, 136, 140, 150, 180 and 240 min. The CiA content was determined by the high performance liquid chromatography (HPLC) assay procedure as described in our previous study.[14] The pure CiA powder, 10 mg, was also used for the dissolution study with both JP 1st and 2nd fluids. All dissolution experiments were carried out triplicate and were highly reproducible. Therefore, only the mean values are given in this report.

Availability Study Four male Wistar rats, weighing 400 to 500 g, were used for each CiA preparation. The rats were fasted overnight but had free access to water. Under anesthesia by an intraperitoneal 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 Bollman *et al.*[15] was used for the collection of lymph from the thoracic lymph duct. 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 to seal it and to fix the cannula in place. After collecting blank blood and lymph samples, CiA preparations weighed into dry tubes (i.d., 3.5 mm; o.d., 3.7 mm, 50 mm length) were inserted into the rat gut through an incision, 0.5 mm, and 0.5 ml of 0.1 N HCl solution was injected to flush them into the stomach. After administration, the gastric incision was sutured and sealed with tissue cement. The oral CiA dose was 7 mg/kg of rat body weight. The continuous output of the lymph from the thoracic lymph duct was collected in hourly fractions in tared culture tubes for 6 h, and their volumes were determined gravimetrically. Single blood sample (100—200 μl) were also obtained at 1, 2, 3, 4, 5 and 6 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 aliquot of the oily solution, 100 mg/ml, per kg of rat body weight was directly injected into the rat stomach with a microsyringe and the hole made was sealed with a drop of tissue cement. Between samplings, the cannula was filled with heparinized saline to maintain its potency. 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.[14] After defrosting of the plasma or lymph samples, 100 to 200 μl aliquots of the samples were used for the CiA assay after extraction into diethyl ether. The extraction procedure was the one we reported before.[14] Briefly, after washing of the residue of the ether extract with hexane, CiA 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 Chemcosorb RP-18 5 μm (25 × 4.6 cm, Chemco Scientific Co., Ltd., Osaka, Japan). The ultraviolet (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). CiA and CiD (used as an internal standard) were detected at 205 nm. Under these conditions, the retention times were 7 min for CiA and 9.5 min for CiD. No interfering peak was detected in the plasma or lymph samples used as blanks or in those from rats given CiA. The concentration of CiA in the biological fluids was determined from calibration curves of peak area ratios of CiA to CiD. The standard curves of CiA added to the rat 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 CiA was estimated as the percentage amount of CiA transferred to the thoracic lymphatics up to the end of the experiment, 6 h, after the oral administration of CiA to rats. As our previous report suggested that the amount of CiA transferred from the systemic circulation into the thoracic lymphatics is negligible,[13] the percentage recovery of CiA in the thoracic lymphatics may be a reasonable index for lymphatic availability of CiA.

Results

Dissolution Behavior of CiA from Preparations As the pulverized preparations containing the equivalent of 10 mg of CiA did not dissolve in 500 ml of the JP 1 st fluid (pH 1.2), CiA was not detected in the test medium for 2 h after initiation of the dissolution study. Therefore, the result is not shown in the figure. In contrast, CiA was detected quickly after the initiation of the study with the conventional olive oil solution, Sandimmun, as shown in Fig. 1. Consequently, the dissolution study was performed using JP 2 nd fluid (pH 6.8) and the dissolution curves of CiA from various pulverized preparations are also shown in Fig. 1. The dissolved amount of CiA in the medium reached 80% of the final value within 8 min. In contrast, CiA was not detected in the 1 st or 2 nd test medium after pure CiA powder was introduced into the dissolution test system.

Lymphatic Availability of CiA from Preparation As a control, 70 μl/kg of Sandimmun was injected into the rat

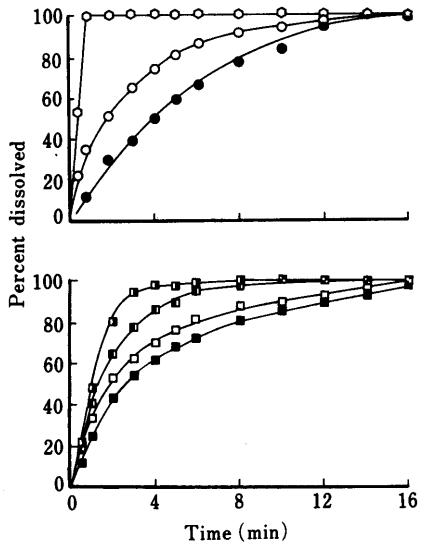

Fig. 1. Dissolution Behavior of Fine Granules Containing 10 mg of CiA in 500 ml of JP XI Test Fluids 1 and 2 at 37 °C

○, Sandimmun; ○, CAP; ●, Eudragit. □, HP-55 #1; ◨, HP-55 #2; ◧, HP-55 #3; ■, HP-55 #4. The dissolution curve for Sandimmun was obtained with JP 1st test medium (pH 1.2), and the others are with JP 2nd test medium (pH 6.8).

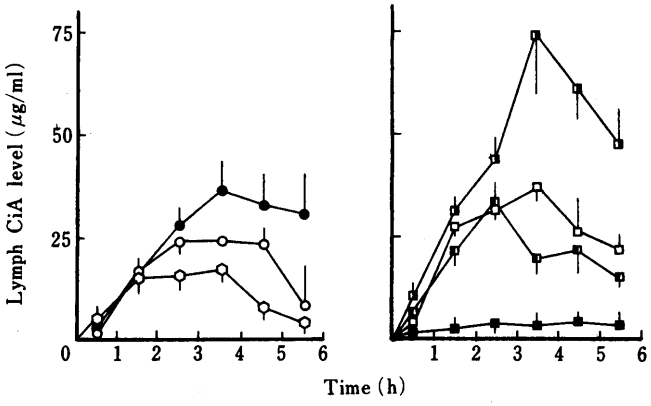

Fig. 2. Lymphatic Concentration of CiA after Intrastomach Administration of Enteric Solid Dispersion of CiA, 7 mg/kg

Each point is the mean ± S.E. of four individual determinations. The symbols are the same as in the caption of Fig. 1.

stomach with a microsyringe at the dose level of 7 mg/kg; the peak lymph CiA level was 18.86±2.56 μg/ml (Fig. 2.) and the percentage transferred into the lymphatics in 6 h was 0.78±0.11% (Table II). When CiA was administered as an enteric pulverized preparation, more CiA was delivered into the lymphatics at higher concentrations as compared to Sandimmun, except in the case of the HP-55 #4 preparation. The HP-55 #2 preparation showed the highest lymphatic availability, namely the peak lymph CiA level was 76.80±12.86 μg/ml, and the percentage transferred into the lymphatics in 6 h was 1.98±0.10% of the administered dose. The other enteric preparations, CAP and Eudragit, did not deliver more CiA into the lymphatics than the optimum HP-55 preparation. Thus, the lymphatic CiA availability was dependent on both the quality and the quantity of the enteric materials.

Systemic Availability of CiA Plasma CiA levels were also dependent on the preparations, as shown in Fig. 3. The mean peak plasma CiA level (C_{max}) from Sandimmun was 0.46±0.10 μg/ml. CAP preparation and Eudragit preparation showed higher peak plasma CiA levels than Sandimmun, 0.71±0.02 and 0.75±0.04 μg/ml, respectively. In addition, higher peak plasma CiA levels, 0.62±0.24 μg/ml (HP-55 #1), 0.99±0.20 μg/ml (HP-55 #2), 0.92±0.05 μg/ml (HP-55 #3) and 0.39±0.15 μg/ml (HP-55 #4), were obtained after intrastomach administration of each HP-55 preparation. The molar ratio of the two components, HCO-60 and HP-55, also affected the plasma CiA levels.

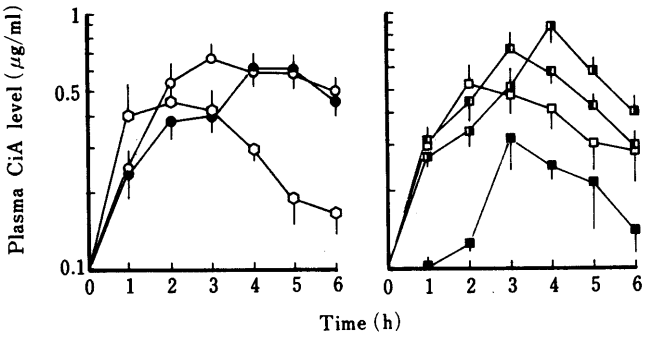

Fig. 3. Plasma Concentration of CiA after Intrastomach Administration of Enteric Solid Dispersion of CiA, 7 mg/kg

Each point is the mean±S.E. of four individual determinations. The symbols are the same as in the caption of Fig. 1.

TABLE II. Lymphatic Delivery of CiA in Rats

Preparation	Peak lymph level (μg/ml)	Percentage of CiA transferred over experimental period[a] (% of dose)	Lymph flow (ml/h)
CAP	38.50±12.23[b]	0.80±0.14	0.25±0.06[b]
Eudragit	28.54± 8.49[b]	0.87±0.29	0.30±0.03
HP-55 #1	43.41±15.05[b]	1.50±0.78[b]	0.27±0.03[b]
HP-55 #2	76.80±12.86[b]	1.98±0.10[b]	0.27±0.05[b]
HP-55 #3	38.43± 1.44[b]	1.13±0.16[b]	0.32±0.03
HP-55 #4	4.86± 1.69[b]	0.11±0.05[b]	0.28±0.01[b]
Sandimmun	18.86± 2.56	0.78±0.11	0.33±0.01

a) Lymph was collected from the thoracic lymph duct over 6 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.

Discussion

In recent years, drug delivery technology has made some exciting strides beyond the traditional concept of pharmaceutics. We have been applying the new concepts and technology to develop a new dosage form for CiA with which the lymphatic delivery of CiA would be improved. In previous studies, we found out that a well-solubilized CiA solution is the best formulation among many other test formulations containing Sandimmun to deliver more CiA into the lymphatics.[7-10] However, many problems, such as stability and difficulty of transportation, are associated with the use of solutions. The best way to overcome these problems would be to develop a pulverized solid oral dosage form for CiA.

One of the approaches to improve the bioavailability of water-insoluble drugs is the use of solid dispersion systems.[16-20] Sugimoto *et al.* made solid dispersions of nifedipine with pharmaceutical additives such as polyvinylpyrrolidone (PVP), hydroxypropylmethylcellulose (HPMC), polyoxy 40 stearate (POS) and HP-55.[16] Their solid dispersion systems were composed of only two components, namely drug and pharmaceutical additive. However, in our case, we must consider one more component, HCO-60, a semi-synthetic surface-active agent. HCO-60 must be used in our preparation to deliver more CiA into the lymphatics. HCO-60 is solid at room temperature, but easily melts as the temperature increases to 50 °C. The most important point is how to pulverize HCO-60 in our solid dispersion system composed of three components. Several combinations of molar ratio of HP-55 to HCO-60 were tested, but in most cases, satisfactory results were not obtained, *i.e.*, a dried powder could not be obtained. Therefore, most of the work was aimed of finding the optimum composition of the solid dispersion system.

Though the solid dispersion system was directly administered into the rat stomach in this report, this system would ultimately be employed as a capsule or an enteric tablet. In the case of a tablet, however, the manufacturing process would become more complicated, because a coating process must be considered. In addition, it is difficult to carry out a bioavailability study using tablet in small animals. Therefore the capsule is thought to be a better formulation for the purpose of the evaluation of our new formulation in small animals. We are evaluating this new dosage form in rabbits, and the results will be published in the near future.

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