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Lymphatic transport of cyclosporin A from the abdominal cavity

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Abstract

The lymphatic transport of cyclosporin A (CyA) has been studied after administration into the abdominal cavity of rats. Two abdominal administration methods were used. One was via a conventional intraperitoneal (i.p.) injection, the other by direct application on the surface of the thoracic lymph duct. To compare the lymphatic transport characteristics, intravenous (i.v.) CyA administration was also performed. The CyA dose was 5 mg/kg in all the experiments. In order to avoid contamination, thoracic lymph fluid was collected through a cannula into the left venous angle. After i.p. administration, the maximum lymphatic transport rate of CyA was determined to be 0.68 ± 0.013 (SD) μg/h, although a higher rate (1.77 ± 0.56 μg/h) was obtained after i.v. injection. By expressing the results as the percentage transferred into the thoracic lymph over a period of 24 h, the percentage amount of CyA transferred after i.p. administration was $0.10 \pm 0.12\%$ which is about 1/4 as compared to i.v. injection, $0.40 \pm 0.012\%$. The efficiency of lymphatic absorption was dependent on the formulation in the preparation applied directly on the thoracic lymph duct. When an oily CyA solution absorbed in gauze was applied, the efficiency of lymphatic absorption ($0.16 \pm 0.007\%$) was almost the same as that observed with i.p. administration. However, when pharmaceutical additives such as polyoxyethylated castor oil derivative (nonionic surfactant, HCO-60) and saturated fatty acid (C_{12}, C_{14}, C_{16} and C_{18}) triglyceride (Pharmasol B-115) were added to the preparation, the efficiency of lymphatic absorption recovered to the level of i.v. injection ($0.37 \pm 0.17\%$).

Key words: Cyclosporin A; Lymphatic transport; Intraperitoneal injection; Thoracic lymph duct; Nonionic surfactant; Saturated fatty acid triglyceride; Lymph level; Rat

1. Introduction

Cyclosporin A (CyA) is a strong immunosuppressant (Wenger, 1981) and is used extensively in the field of organ transplantation (Jones et al., 1989; Kahan, 1989). The pharmacological mechanism of CyA is described as interfering with the process of T cell activation by blocking transcription initiation of interleukin-2 (Kronke et al., 1984; Tocci et al., 1989). Therefore, the target cell of CyA is thought to be T cells, especially helper T cells (Baumann et al., 1992; Sigal et al., 1992). As these lymphocytes exist mostly in the lymphatic system in the body, we made an assumption that the immunosuppressive effect of CyA is related to its concentration in the lym-

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phatic system (Takada et al., 1985a,b, 1986a,b, 1986, 1987, 1988). According to this assumption, an oral solid dispersion system from which CyA is delivered into the lymphatic system was developed. (Takada et al., 1989a,b). Before preparing such an oral solid CyA delivery system, this CyA system was evaluated as an oral solution by mouse skin allogenic transplantation (Takada et al., 1986a,b) and rat allogenic heart transplantation experiments (Yasumura et al., 1986; Nakaji et al., 1988), In these experiments, significant prolongation of the survival of allografts was observed as compared to the conventional oily CyA solution.

In the circulation system, most of the lymphocytes pass through the thoracic lymph duct several times a day (O'Driscoll, 1992). Therefore, if a new dosage form from which CyA directly penetrates into the thoracic lymph duct is made, the therapeutic efficiency of CyA should be improved further. As the direct injection of CyA solution into the thoracic lymph duct is not practical, there is a need to enhance the penetration of CyA into the thoracic lymph duct. Apart from the arterial and venous vessels, the lymph duct is generally thin and erodible. Therefore, in this report, the possibility of increasing the penetration of CyA into the thoracic lymph duct from the abdominal cavity has been studied in rats.

2. Experimental

2.1. Materials

Cyclosporin A (CyA) and D (CyD) powders were kindly supplied by Sandoz Co., Ltd (Basel, Switzerland). Sandimmun® i.v. oily solution (CyA concentration 50 mg/ml) was obtained from Japan Sandoz Co., Ltd (Tokyo, Japan). Polyoxyethylated castor oil derivative (HCO-60®) was a gift from Nikko Chemicals Co., Ltd (Tokyo, Japan). Saturated fatty acid (C_{12}, C_{14}, C_{16} and C_{18}) triglycerides (Pharmasol® B-115) were also a gift from Nippom Oil & Fats Co., Ltd (Tokyo, Japan). HCO-60 and Pharmasol B-115 were used without further purification. All other reagents were commercial products of reagent grade.

2.2. Test dosage forms

For i.v. and i.p. injections, CyA i.v. solution (50 mg/ml) was used. For the thoracic lymph duct application of CyA using 300 g rats, 30 μl of CyA i.v. solution was absorbed on 10 mg of gauze which was shaped as a pole of 2 mm o.d. × 15 mm length. A CyA implant in which Pharmasol B-115 was used as a base was prepared as follows. 5 mg of CyA powder and 20 mg of HCO-60 were dissolved in 300 mg of Pharmasol B-115 at 60°C. After pouring the mixture into a mold (2 mm i.d × 10 cm), the mold was placed in a freezer (4°C) and an implant was made. This implant was cut into several parts.

2.3. Lymphatic transport study

Four male Wistar rats (weight 300–350 g) were used for each experiment. The rats were fasted overnight but had free access to water. The rats were anesthesized by an intraperitoneal (i.p.) injection of sodium pentobarbital (45 mg/kg). A modification of the method of Ellis (1966) was used for the collection of lymph fluid from the left 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. During the collection of blank lymph samples, surgical operations were performed for the administration of CyA. To group A rats, CyA was administered by intravenous (i.v.) injection. A 100 μl aliquot of the oily solution (50 mg/ml) per kg of rat body weight was directly injected into the femoral vein. After injection of CyA (5 mg/kg) the incision was sutured. For i.p. injection of CyA solution to group B rats, abdominal incision was performed. CyA solution was administered into the abdominal cavity (5 mg/kg). After administration, the incision was also sutured. To the other two groups of rats (C and D), CyA preparations were applied directly on the surface of the thoracic lymph duct. After application of the CyA implant, the abdominal incision was sutured. The CyA dose was 5 mg/kg in all of the

groups. After administration of CyA, the output of lymph from the thoracic lymph duct was collected in 1-h fractions in tared culture tubes until 8 h and thereafter to 24 h. The volumes of the each lymph sample was determined gravimetrically. All the lymph samples were immediately frozen in a deep freezer at −20°C until analysis.

2.4. Drug assay

The analytical method used was basically similar to the HPLC assay method developed by Shibata et al. (1987). After defrosting of the lymph samples, 100 μl of dextran solution (0.1 mM sodium dextran sulfate) were added to 100 μl aliquots of the samples and were used for the CyA assay after extraction into *n*-hexane. The extraction procedure has been reported previously (Takada et al., 1985a,b; Shibata et al., 1987). Briefly, the hexane phase was washed with 0.5 N HCl, the following CyA in that phase was extracted into methanol and the drug was re-extracted 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 under a stream of nitrogen gas at 60°C. The resulting residue was dissolved in 200 μl of the mobile phase. An aliquot of 100 μl was then injected onto the column. A Shimadzu LC-3A pump and a Shimadzu SIL-6A automatic sample injector were used for chromatographic analysis. The analytical column was a Lichrosorb Si-60 column (5 μm, 25 × 0.46 cm; Chemco Scientific Co., Ltd, Osaka, Japan). The UV detector was a Shimadzu SPD-10A. The column was maintained at 60°C with a column heater. The mobile phase was composed of *n*-hexane:ethanol (85:15) and the flow rate was 1 ml/min (50 kg/cm^2). CyA and CyD (used as an internal standard) were detected at 205 nm. Under these conditions, the retention times were 6.5 min for CyD and 7.5 min for CyA. No interfering peak was detected in the plasma or lymph samples used as blanks or in those from rats 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 rat lymph samples were linear over the range of 0.1–20 mg/ml and passed through the origin.

2.5. Estimation of lymphatic availability

The lymphatic availability of CyA was estimated as the percentage amount of CyA transferred to the thoracic lymph duct up to the end of the experiment (24 h) after the administration of CyA preparations to rats.

2.6. Analysis of data

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).

3. Results and discussion

The lymphatic CyA transport rate vs time curves for the four groups of rats are shown in Fig. 1. The peak lymphatic transport rate of CyA in the i.v. injected rats (group A) was 1.77 ± 0.56 μg/h during the 1–2 h interval. As compared to

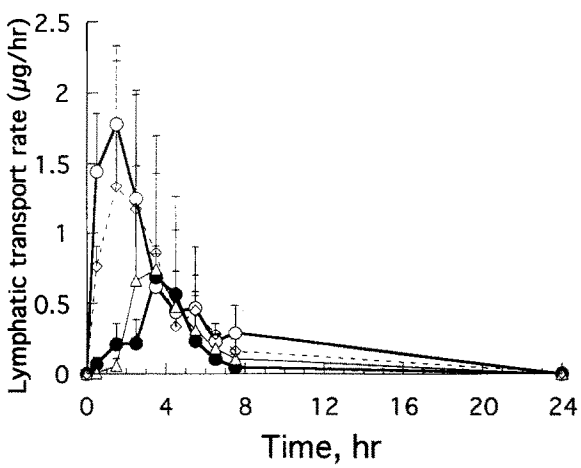

Fig. 1. Lymphatic transport rate vs time curves of CyA after different administration modes at the dose level of 5 mg of CyA/kg of rat body weight. (○) i.v. injection, (●) i.p. injection, (△) direct application onto thoracic lymph duct, (◇) implant. Each point is the mean of four individual determinations and is expressed as the mean ± S.D.

the case of i.v. injection, the peak lymphatic transport rate after i.p. administration appeared at a later phase (3–4 h interval) due to the process of absorption from the abdominal cavity into the lymphatics. In the same groups of rats, CyA transfer into the blood circulation was examined as a preliminary experiment. For the sake of experimental condition, the cannulation into the thoracic duct could not be performed in these animals. With respect to the plasma samples for 6 h, no CyA was detected with our HPLC assay method. Therefore, the evaluation of CyA transport from the administered site was performed with thoracic lymph levels. Fig. 2 depicts the time course of the cumulative amount of CyA transferred into the thoracic lymph duct for 24 h. The ordinate is represented as the percentage amount of the administered CyA transferred into the thoracic lymph duct in all the groups of rats. The percentage of CyA transferred into the thoracic lymph duct over the 24 h period in group A rats (i.v. injection) was 0.40 ± 0.012 and $0.10 \pm 0.12\%$ in group B rats (i.p. injection), respectively. When CyA was directly applied onto the surface of the thoracic lymph duct, the lymphatic transport efficiency was dependent on the preparations. In group C rats, which received the CyA solution absorbed on gauze, the peak lymphatic transport rate of CyA was 0.73 ± 0.12 μg/h during the 3–4 h inteval (Fig. 1) Furthermore, the percentage of CyA transported into the thoracic lymph duct over the 24 h period was decreased to $0.16 \pm 0.012\%$ (Fig. 2). However, when CyA was administered as an implant containing HCO-60 and Pharmasol B-115, the percentage of CyA transported into the thoracic lymph duct over the 24 h period recovered to $0.37 \pm 0.17\%$, which is almost the same as that observed in group A rats (i.v. injection). The maximum transport rate was 1.33 ± 0.25 μg/h during the 1–2 h interval (Fig. 1). The peak time differences were observed between group C and D, or B and D. As Pharmasol B-115 dissolves rapidly at body temperature (37°C), CyA was thought to be released faster in group D rats than in the other groups, B and C.

CyA is a valuable immunosuppressant in transplantation immunology, increasing the survival of transplants by inhibiting the T lymphocytes responsible for rejection (Rynasiewicz et al., 1982; Cohen et al., 1984). However, CyA exerts a number of side effects on kidney, central nervous system and liver (Awni, 1992). To reduce the side effects and increase the pharmacological activity of CyA, the concept of local immunosuppression has been proposed (Gruber, 1992). He suggested that utilization of a local drug administration system would elicit a concomitant reduction in systemic drug exposure and reduce the side effects of CyA as CyA belongs to a class of nonspecific immunosuppressants. Our goal of delivering more CyA into the lymphatics where its target cell, helper T cells, has a greater probability of existing than in the central circulation, would certainly be of value in achieving local immunosuppression. During the early post-operative period after transplantation, i.v. therapy is generally performed. However, the lymphatic delivery of CyA from the conventional i.v. oily solution is low, less than 1.0% (Ueda et al., 1983). Therefore, we have tried to improve the lymphatic delivery of CyA by direct application onto the surface of the thoracic lymph duct. However, both the peak lymphatic transport rate and the percentage transferred into the lymph after administration into the abdominal site were lower than those obtained after the i.v. CyA injection.

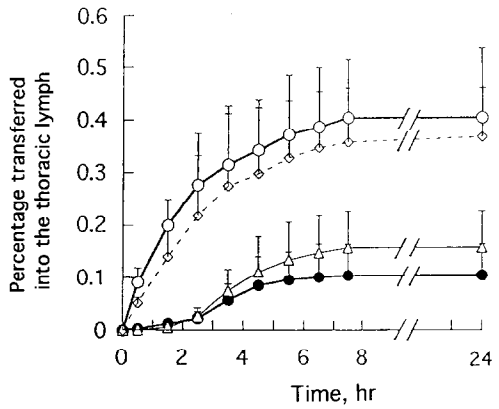

Fig. 2. Cumulative percentage of CyA transported into thoracic lymph duct after different administration modes at the dose level of 5 mg of CyA/kg of rat body weight. (○) i.v. injection, (●) i.p. injection, (△) direct application onto thoracic lymph duct, (◇) implant. Each point is the mean of four individual determinations and is expressed as the mean ± S.D.

As compared to the arterial and venous vessels, the lymph duct is thin. Therefore, we believe that the penetration of CyA from outside into the thoracic lymph duct could be improved with ease.

In general, the pharmacological activity of immunosuppressants is thought to be defined by the integral of the amount of drug exposed by the helper T cells with respect to time (Grevel, 1992). Therefore, there remains the possibility of achieving local immunosuppression in the thoracic lymph duct by increasing the time of exposure of helper T cells to CyA. As a next step, we are currently attempting to prepare a sustained release CyA preparation for direct application to the thoracic lymph duct.

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