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Report

Enhanced Selective Lymphatic Delivery of Cyclosporin A by Solubilizers and Intensified Immunosuppressive Activity Against Mice Skin Allograft

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The absorption and lymphatic delivery of a new immunosuppressive drug, cyclosporin A (CsA), were studied in rats by administering CsA orally after solubilization with HCO-60 (polyoxyethylated hydrogenated castor oil), sugar ester, and oils. After the administration of solubilized CsA (7 mg/kg) to rats with thoracic lymph duct cannulas, both plasma and lymph CsA levels were measured over 6 hr. The lymph CsA levels were strongly affected by the solubilizers. The rank order of the solubilizers in enhancing lymph absorption was HCO-60 (57 μg/ml) > sugar ester (46 μg/ml) > sesame oil (3.5 μg/ml) > linoleic acid (0.4 μg/ml), where the parentheses show the maximum lymph CsA levels. Plasma CsA levels were below 2 μg/ml in each group of animals and were barely altered by the solubilizers. These results support the selective lymphatic delivery of CsA with solubilizers such as HCO-60 and sugar ester. The immunosuppressive activity of CsA (1 mg/kg) solubilized with HCO-60 was nearly equivalent to the sesame oil solution with 7 to 15 mg/kg CsA in the skin-allograft mice model.

KEY WORDS: cyclosporin A; lymphatic delivery; immunosuppressive activity.

INTRODUCTION

Clyclosporin A (CsA), a cyclic undecapeptide with a molecular weight of 1201, is clinically effective in inhibiting graft rejection in renal, hepatic, cardiac, lung, pancreatic, and bone marrow transplantations (1). It is highly lipophilic and virtually insoluble in water (2). Because of these properties, an olive oil solution of CsA is clinically used as an oral dosage form (3). In view of both the lipophilic property of CsA and the oily dosage form, one might expect that the intestinal lymphatic absorption of CsA would be extensive. However, studies in rats showed that only 0.35 to 0.47% of the oral CsA dose is lymphatically absorbed for up to 114 hr, while the systemic availability of CsA was 21.3% (4). Borel and Wiesinger suggested that the immunosuppressive activity of CsA is related to a selective action against T lymphocytes, which play a central role in the induction of immune responsiveness (5). As the lymphocytes circulate mainly in the lymphatic system in the body, the immunosuppressive activity of CsA is thought to be dependent on the drug concentrations in the lymphatic system (6).

We succeeded in the selective lymphatic delivery of drugs such as bleomycin (7), interferon (8), and 1-hexylcarbamoyl-5-fluorouracil (HCFU) (9) by administering these drugs in a lipid-surfactant mixed micellar (MM) solution into the large intestine or peritoneal cavity of rats. In our first study, MM solution was also used as a promoter for the selective lymphatic delivery of CsA, and considerably higher lymph CsA levels were obtained (10). In addition, we also studied the effect of the administration route on the lymphatic delivery efficiency of CsA with MM solution, and it was confirmed that the oral route is more efficient than the rectal or intraperitoneal route (11). However, in subsequent studies we found more efficient promoters such as HCO-60 and sugar ester for the selective lymphatic delivery of CsA. In addition, the immunosuppressive activity of CsA in the two dosage forms (i.e., HCO-60 solution and oily solution) was studied using the mice skin-allograft model to determine whether the immunosuppressive activity of CsA is dependent on its lymphatic concentration.

MATERIALS AND METHODS

CsA was kindly supplied by Sandoz Ltd., Basle, Switzerland. Linoleic acid of the 99.0% purity grade was obtained from Nippon Oil & Fats Co. (Tokyo) and HCO-60 (polyoxyethylated, 60 mol, hydrogenated caster oil) was obtained from Nikko Chemicals Co. (Tokyo). Sugar ester (DK ester F-140) was obtained from San-ei Chemical Industries Ltd. (Toyonaka, Japan). Sesame oil was obtained from Wako Pure Chemicals (Osaka, Japan). All other chemicals were of reagent grade commercially obtained.

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Preparation of Test Solution

The oily solution (sesame oil and linoleic acid) was prepared by dissolving CsA in the oil. HCO-60 or sugar ester solution was prepared by dissolving CsA in 8% (w/v) HCO-60 or 0.2% (w/v) sugar ester solution, followed by sonication at 25°C for 5 min with an Ohtake 5202 sonicator (Tokyo). MM solution was prepared by dispersing linoleic acid [final concentration, 5.0% (w/v)] containing CsA and HCO-60 [final concentration, 8.0% (w/v)] in distilled water, followed by sonication at 25°C for 5 min. The final CsA concentration was 3.5 mg/ml in each test solution.

Animal Preparation

Male Wistar rats weighing 350–400 g were used. Four to six rats were used for each experimental group. The rats were fasted overnight but had free access to water. Under anesthesia by 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 at various times. A modification of the method of Bollman *et al.* (12) was used for the collection of lymph from the thoracic duct. The thoracic duct was cannulated with a heparin-filled flexible vinyl catheter (i.d., 0.5 mm; o.d., 1.2 mm; Dural Plastics) and was fixed with a drop of tissue cement (Aron Alpha, Sankyo Co., Tokyo).

Drug Administration and Collection of Blood and Lymph Samples

At 1 hr before the administration, 1 ml of fresh milk was orally administered to make the lymph flow at higher levels and to facilitate the cannulation. After collecting blank blood and lymph samples, 1 ml of each CsA test solution/500 g of rat body weight was orally administered to each group of the rats, corresponding to a CsA dose of 7.0 mg/kg per animal. After dosing, the continuous output of lymph from the thoracic duct was collected in hourly fractions in tared culture tubes for 6 hr and their volumes were determined gravimetrically. Single blood samples (100–200 μl) were also obtained on an hourly basis in heparinized tubes through the cannula, but they were staggered to coincide with the midpoint of the lymph collection intervals (i.e., 30, 90, and 150 min, etc.). Between samplings, the cannula was filled with heparinized saline to maintain its patency.

Drug Assay

The plasma concentration of CsA was determined with a high-performance liquid chromatographic procedure previously reported in our laboratory (13). As Follath *et al.* have reported, a marked temperature dependence of drug partitioning between red cells and plasma (14), plasma was separated at 37°C from red cells immediately after the collection of rat blood. Fifty to one hundred microliters of the plasma or lymph sample was used for the CsA assay. All values are expressed as the mean ± SE.

Survival Study of Skin Allograft in Mice

Fitted pinch grafts of skin from male C57BL/6(H-2^b) donor mice were transplanted to adult male BALB/c(H-2^d) recipients according to the method of Billingham and Medawar (15). The mice were separated into five groups and each group contained six mice. Graft survival rate was determined by daily observation, the criterion for rejection being epithelial survival. As an oily dosage form, CsA was dissolved in sesame oil at various concentrations, which permitted the dose, 1, 7, and 15 mg/kg, to be given in a fixed volume of 0.1 ml/10 g body weight. As a representative dosage form targeted for lymphatic delivery, HCO-60 solution was used with a CsA dose of 1 mg/kg. CsA was administered once daily by gavage from day 1 to day 12. Control rats received the same volume of sesame oil.

Statistics

With respect to both plasma and lymph CsA levels, statistical analysis was performed using the unpaired *t* test.

RESULTS

The concentration–time profiles of CsA in rat plasma and lymph after oral administration of CsA in different solubilizer preparations are shown in Fig. 1. As the rat lymph samples were collected in hourly fractions, the mean lymph CsA concentrations are plotted at the midpoint of the each collection interval. According to a previous report on the lymphatic availability of CsA after oral dosing in an olive oil solution (4), maximum lymphatic CsA delivery was reached within 6 hr after administration. Therefore, collection of the biological samples was performed over 6 hr in this study. In contrast to the plasma levels, lymph CsA levels were greatly

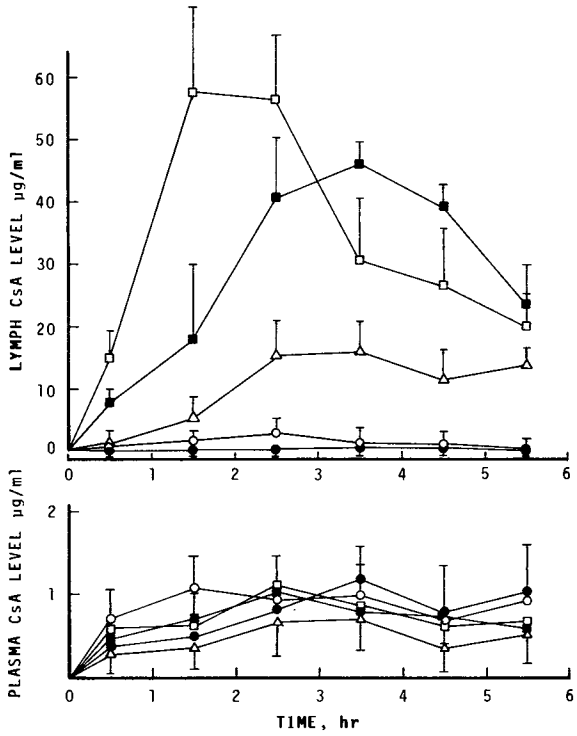

Fig. 1. Concentrations of CsA in the plasma and in the thoracic duct lymph after the oral administration of CsA, 7 mg/kg, prepared with five solubilizers. (—□—) HCO-60 solution; (—■—) sugar ester solution; (—△—) MM solution; (—○—) sesame oil; (—●—) linoleic acid. Each point represents the mean ± SE.

Table I. Lymphatic Delivery of CsA in Rats[c]

Solubilizer	Cumulative amount of CsA over 6 hr (% of dose)	Lymph flow (ml/hr)	Lymph/plasma ratio
Sesame oil	0.19 ± 0.04	0.508 ± 0.028	2.71 ± 1.82
Linoleic acid	0.05 ± 0.62[a]	0.363 ± 0.018	0.84 ± 0.38[b]
MM solution	0.66 ± 0.19[a]	0.292 ± 0.007[a]	25.0 ± 12.7[a]
HCO-60 solution	2.14 ± 0.04[a]	0.347 ± 0.031[b]	85.1 ± 14.9[a]
Sugar ester solution	1.62 ± 0.92[a]	0.313 ± 0.057[b]	79.2 ± 18.1[a]

[a] Statistically significant difference from the sesame oil experiment by Student's t test ($P < 0.01$).
[b] Statistically significant difference from the sesame oil experiment by Student's t test ($P < 0.05$).
[c] Each value represents the mean ± SE.

affected by the solubilizers. While the MM solution produced considerably higher lymph CsA levels than oily solutions in our previous report (10), the HCO-60 solution yielded the highest lymph CsA levels, approximately twofold higher than the MM solution. The sugar ester preparation also produced higher lymph CsA levels than the MM solution. By multiplying the lymph CsA levels by the lymph flow, we obtain the lymphatic delivery rate of CsA. As shown in Table I, lymph flow was decreased by 30 to 40% with the use of the solubilized dosage forms such as HCO-60 and sugar ester solution, compared to the sesame oil solution. However, the enhancing effects of these solubilizers on the lymphatic CsA concentration is extensive; therefore, the solubilizers HCO-60 and sugar ester considerably enhanced the availability of CsA over that achieved with the other preparations. Table I also shows the effect of solubilizers on the extent of lymphatic availability of CsA over 6 hr after oral administration. Even with respect to the extent of lymphatic CsA availability, the same rank order was obtained for the effect of solubilizers. In summary, the rank order of the promoting effect of solubilizers on the selective lymphatic delivery of CsA is as follows: HCO-60 > sugar ester > MM solution > oily solutions. On the other hand, differences with respect to the CsA plasma levels were minimal, contrary to our expectation.

To examine the effect of solubilizers on the pharmacological activity of CsA, the skin-allograft survival rate was studied with mice, and preliminary results are presented in Fig. 2. The graft survival rate at 12 days was greatest in group D (sesame oil, 15 mg/kg, group). However, the graft survival rate of group E (HCO-60, 1 mg/kg, group) was between that of group D and that of group C (sesame oil, 7 mg/kg, group).

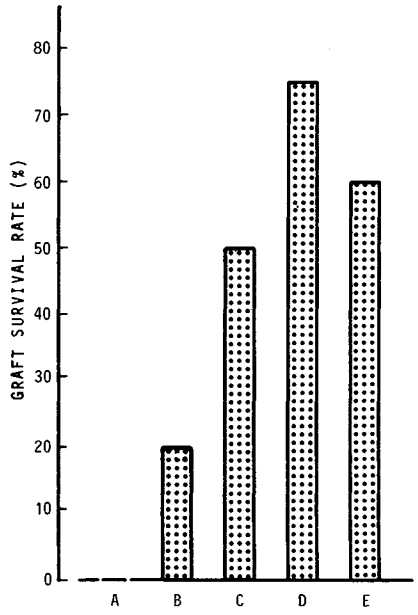

Fig. 2. Effect of solubilizers on the skin-allograft survival rate at the 12th day after initiation of CsA therapy to mice. Each group consists of six mice. Groups A, B, C, and D received CsA dissolved in sesame oil at doses of 0, 1, 7, and 15 mg/kg, respectively. Group E received CsA, 1 mg/kg, solubilized with HCO-60.

DISCUSSION

Clinically, CsA is administered as an olive oil solution to patients undergoing organ transplantation or having graft-versus-host disease (GVHD). However, this regimen is associated with considerable toxicity to the kidneys (16) and liver (17). The most probable cause of these toxicities is the high clinical dose of CsA, 5 to 10 mg/kg. The reason for such a high dose requirement is the low bioavailability of CsA, namely, 4 to 26% in human patients (18). However, this bioavailability is defined as the extent to which the drug is absorbed from the administered site and reaches the systemic circulation (19). Most clinicians pay attention to the blood or plasma CsA level, and its determination has become a routine clinical assay in immunosuppressive therapy with CsA. Thus the CsA dose should be adjusted not to exceed its trough level of 200 ng/ml (20). However, the target cell of CsA is the helper T cell, which circulates in the lymphatic system. Therefore, the major target site of CsA may be the lymphatic system. However, with respect to the lymphatic delivery of CsA administered orally, we must consider the following two consecutive processes: (i) the absorption from the gastrointestinal (GI) tract to the intestinal epithelial cells and (ii) the transfer from the epithelial cells into the mesenteric lymph. For the latter process with highly lipophilic compounds such as fatty acids, two separate pathways have been postulated by Tso *et al.* (21), namely, the chylomicron (CM) transport system and the very low-density lipoprotein-sized (VLDL) particles transport system. Linoleic acid is absorbed from the GI tract and is used for the resynthesis of triglycerides, phospholipids, and cholesterol esters, followed by transport into the lymphatics in the form of CM (22). Therefore, in the previous studies (10), we used linoleic acid as a component of MM solutions. On the other hand, the surfactant, HCO-60, was used as an absorption promoter. However, the lymphatic delivery of CsA was more enhanced by dissolving CsA in HCO-60 solution than in MM solution. The other surfactant, sugar ester, also enhanced the lymphatic delivery of CsA. When CsA dissolved in HCO-60 solution was administered to three rats preadministered 1 ml saline instead of fresh milk, no significant difference was observed (data not shown). Since saline

preadministration fails to stimulate CM formation, this result argues against the hypothesis selective lymphatic delivery of CsA is due to the interaction of CsA with CM. As HCO-60 and sugar ester are representative absorption promoters (23,24), the absorption of CsA from the GI tract into the epithelial cells was thought to be enhanced. If we assume that this absorption-promoting effect of HCO-60 and sugar ester is the only cause of the selective lymphatic delivery of CsA, the plasma CsA levels would increase proportionally to the increase in the lymph CsA levels. However, the plasma CsA levels were largely independent of the solubilizers. Therefore, the mechanism of the enhanced lymphatic delivery of CsA by solubilizers remains to be resolved.

To examine whether the pharmacological activity, i.e., immunosuppressive activity, of CsA is related to its lymphatic concentration, the skin-allograft survival rate was measured with mice. Rats were not selected for these preliminary experiments because of experimental difficulties with the rat organ transplant model. The present result shows that the immunosuppressive activity of oral CsA, 1 mg/kg, solubilized with HCO-60 is nearly equivalent to that of the sesame oil solution with 7–15 mg/kg CsA. On the basis of this result, we are now studying the immunosuppressive activity of oral CsA in HCO-60 solution as compared to the conventional oily solutions in the rat heart transplant model.

To reduce the nephrotoxic properties of CsA while retaining immunosuppression, Cunningham *et al.* (25) suggested the use of inducers of the hepatic cytochrome *p*-450 enzyme system. However, our results suggest another possibility to reduce the side effects of CsA, by targeting CsA absorption to the lymph as a potential major site of the immunosuppressive activity of CsA.

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