EFFECT OF ADMINISTRATION ROUTE ON THE SELECTIVE LYMPHATIC DELIVERY OF CYCLOSPORIN A BY LIPID-SURFACTANT MIXED MICELLES

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The absorption and lymphatic delivery of a new immunosuppressive drug, cyclosporin A (CsA), with the aid of lipid surfactant mixed micelles (MM) system using different administration routes were studied in the thoracic duct cannulated rat model at a dose level of 7 mg/kg. The rectal or intraperitoneal (i.p.) administration of CsA indicated a small amount of CsA in the plasma and in the lymph for 6 h. As oral routes, intrastrachomach (i.s.) and intraduodenal (i.d.) administration of CsA were performed and high lymph CsA levels were obtained. The i.s. administration of CsA resulted in the highest CsA levels in the lymph, 16 µg/ml, about twenty times higher than the rectal or i.p. administration. These results strongly support the usefulness of an oral CsA dosage form for the selective lymphatic delivery of CsA in clinical immunosuppressive therapy by means of a new mixed micelles system.

Keywords — cyclosporin A; drug delivery; lymph level; lipid-surfactant mixed micelle; administration route

INTRODUCTION

Cyclosporin A (CsA), a cyclic endopeptide having a molecular weight of 1201, is found to be of increasing clinical usefulness in the inhibition of graft rejection in renal, hepatic, cardiac, lung, pancreatic and bone marrow transplantations. It is extremely lipophilic and virtually insoluble in water. Because of these properties, an olive oil solution of CsA is clinically used as an oral dosage form. In view of both the high lipophilic property of CsA and the oily dosage form, it was reasoned that the intestinal lymphatic absorption of CsA would be extensive. However, basic studies using rats showed that about 0.35 – 0.47% of the oral CsA dose is absorbed lymphatically for up to 114 h though the systemic availability of CsA was 21.3% in these rats. Laupacis 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. In the body, the lymphocytes circulate in the lymph of the lymphatic system. Therefore, the immunosuppressive activity of CsA is thought to be dependent on the concentrations of the drug in the lymphatic system because the lymph is intimately associated with the immune system.

As a preliminary experiment, we used a lipid-surfactant mixed micelles (MM) system for promoting the absorption from the gastrointestinal tract and selective lymphatic transport of CsA and obtained good results. In the case of the administration of CsA in MM system, the lymph CsA level was about 16 times that found with a conventional oily solution after intraduodenal (i.d.) administration to rats. However, i.d. route is clinically unfavorable.

In this paper, therefore, we have tried to examine the effect of several different administration routes, such as intrastrachomach (i.s.), intraduodenal (i.d.), rectal and intraperitoneal (i.p.) routes on the selective lymphatic transport of CsA using a thoracic duct cannulated rat model.

MATERIALS AND METHODS

Materials — CsA was kindly supplied by Sandoz Ltd., Basle, Switzerland. Linolic acid of 99.0% high purity grade (Nippon Oil & Fats Co., Ltd., Tokyo, Japan) and HCO-60 (polyoxyethylated, 60 mol, hydrogenated caster oil, Nikko Chemicals Co., Ltd., Tokyo, Japan) were used as components of MM.

Preparation of Test Solution — MM solution
Enhanced Lymphatic Delivery of CsA

was prepared by dispersing linolic acid (5.0 w/v%) containing CsA and HCO-60 (8.0 w/v%) in the distilled water followed by sonication at 25 °C for 5 min with an Ohtake sonicator, model 5202 (Tokyo, Japan). The final CsA concentration, except for rectal administration, was 3.5 mg/ml MM solution. In the case of rectal administration, the final concentration was 7.0 mg/ml MM solution because of the limited space of the rectum.

Animal Preparation — Male Wistar rats weighing 350–400 g were used. Four rats were used for each experimental group. The rats were fasted overnight but had free access to water. Under anesthesia by i.p. injection of sodium pentobarbital, 32 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. was used for the collection of lymph from the thoracic duct.81 To keep the lymph flow at higher levels and to make cannulation easier, 1 ml of fresh milk was orally administered to each rat. The thoracic ducts were cannulated with a heparin-filled flexible vinyl catheter (i.d. 0.5 mm, o.d. 1.2 mm; Dural Plastics) and fixed with a drop of tissue cement (Aron Alpha®, Sankyo Co., Tokyo, Japan).

Drug Administration and Collection of Blood and Lymph Samples — After collecting blank blood and lymph samples, CsA-MM solution (corresponding to a dose of CsA of 7.0 mg/kg per animal) was given to each group of the rats. For i.s., i.d., and i.p. administrations, 2 ml of CsA-MM solution per kg of rat body weight was directly injected into the stomach, duodenum and peritoneal cavity, respectively. In the case of i.s. or i.d. administration, the opening made in the gastrointestinal tract was closed with a drop of tissue cement just after administration. For rectal administration, a restricted rectal infusion device was used as previously reported by us.9 The device was constructed with two septum plugs connected at a fixed distance, 3 cm, with a stainless needle. This device was inserted into the rat rectum from the anus. The upper septum plug (8.0 mm diameter) was used to prevent the upward spreading of CsA-MM solution. The lower septum plug (10.0-mm diameter) was glued to the anus. A 0.5 ml/mg of CsA-MM solution was injected into the rectum following the withdrawal of a volume of air from the rectum identical to the volume of the rectal CsA-MM solution.

After dosing, the continuous output of lymph from the thoracic duct was collected in hourly fractions in tared culture tubes and their volumes determined gravimetrically. Blood samples, about 400 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, 150 min, etc.). Between samplings, the cannula was filled with heparinized saline to maintain its patency.

Drug Assay — The plasma and lymph concentrations of CsA were determined with a high-performance liquid chromatographic procedure previously reported by our laboratory.10 Briefly, CsA was extracted first with diethyl ether, then with a carbon tetrachloride-70% methanol system and was chromatographed on a microparticulate CN column. The effluent was monitored by ultraviolet (UV) detection at 212 nm. The mobile phase consisted of CH₃CN-H₂O (43:57). Levels were estimated by a chromatographic technique of comparing peak areas obtained from test plasma or lymph samples with standard curves obtained from rat plasma or lymph samples containing added known amounts of CsA. The standard curve of CsA added to the rat plasma or lymph was linear over the range 0.5–50 µg/ml and passed through the origin. Follath et al. reported that there is a marked temperature dependence of drug partition between red cells and plasma.11 Accordingly, plasma was separated at 37 °C from red cells immediately after the collection of rat blood and the plasma was used for CsA assay. About 50–200 µl of the plasma or lymph sample was used for CsA assay. All values are expressed as their mean ± S.E. Statistical analysis was performed using the unpaired t-test.

RESULTS

The concentration–time profiles of CsA both in the rat plasma and lymph after oral administrations, namely i.s. and i.d., of CsA-MM solution is shown in Fig. 1. Although the plasma and lymph CsA levels reached their maximum at 2–4 h after administration, the maximum concentration in the lymph was about twenty times higher than that in the plasma after i.s. adminis-
tration. By comparing the results obtained after the i.d. administration of CsA-MM solution, significant differences were not detected with respect to the peak lymph CsA levels. Similarly, significant differences were not detected with plasma CsA levels for 6 h after i.s. and i.d. administrations. On the other hand, Fig. 2 shows the CsA levels-time profiles both in the plasma and in the lymph after nonparenteral administrations. Only a small amount of CsA was detected in the rat plasma samples and the levels were below 0.5 \( \mu \text{g/ml} \). Therefore, the values were estimated by extrapolating the calibration curve. As shown in this figure, the rectal and i.p. administrations of CsA-MM solution showed considerably lower CsA levels both in the lymph and plasma as compared to the oral administrations. However, the lymph CsA levels after i.p. administration was slightly higher than that obtained after rectal administration. Plasma CsA levels obtained after rectal or i.p. administration were also lower than that obtained after oral administrations. Table 1 shows the amount of CsA delivered into the lymph from the rat gastrointestinal tract for 6 h after different routes of administration. The oral administrations of CsA in MM solution showed about ten-fold more amount of CsA in the lymph than that obtained after rectal or i.p. administration. The mean ratio of CsA in the lymph to that in the plasma corresponding to the midpoint of lymph collection interval (L/P ratio) which was calculated from the data in Figs. 1 and 2 are also shown in the table. The L/P ratios obtained after oral administrations are about four to seven-fold greater than that obtained after rectal or i.p. administration.

DISCUSSION

In our previous report showing the result of a preliminary experiment, the selective lymphatic delivery of CsA was accomplished by administering CsA in MM solution into the rat duodenum. However, this administration route (i.d.) of CsA is impractical in the clinical immunosuppressive therapy after organ transplantation. We also were successful in the selective lymphatic delivery of drugs such as bleomycin, interferon and 1-hexylcarbamoyl-5-fluorouracil (HCU) by administering them in MM solution into the large intestine and/or peritoneal cavity. Attempts were made to administer the CsA-MM solution through nonparenteral routes. However, the selective lymphatic delivery of CsA was not obtained when CsA was administered i.p. or rectally. On the other hand, the i.s. administration of CsA resulted in high lymph CsA levels, almost equal to that obtained after i.d. administration. In addition, no signifi-

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**FIG. 1.** Concentrations of CsA in the Plasma and in the Lymph of the Thoracic Duct after Oral Administration of CsA in MM Solution to Rats, 7 mg/kg
- ○, lymph CsA levels after i.s. administration;
- □, lymph CsA levels after i.d. administration;
- ●, plasma CsA levels after i.s. administration;
- ■, plasma CsA levels after i.d. administration.
Each point represents four individual determinations, and is expressed as the mean ± S.E.

**FIG. 2.** Concentrations of CsA in the Plasma and in the Lymph of the Thoracic Duct after Rectal or Intraperitoneal (i.p.) Administration of CsA in MM Solution to Rats, 7 mg/kg
- ○, lymph CsA levels after rectal administration;
- △, lymph CsA levels after i.p. administration;
- ●, plasma CsA levels after rectal administration;
- ▲, plasma CsA levels after i.p. administration.
Each point represents four individual determinations, and is expressed as the mean ± S.E.
cant difference was detected regarding the cumulative amounts of CsA delivered into the thoracic duct lymph between the two administration routes, i.e. and i.d. In our preliminary experiment, we used the i.d. route for the administration of CsA-MM solution because of the possibility of the loss of the potency of MM solution due to the dilution with gastric juice during passage through the stomach. However, this result suggests that the potency of the MM solution was not decreased by passing through the stomach.

With respect to the mechanism for the selective lymphatic transfer of CsA by MM system, we must consider the following two physiological processes. Namely, (1) the absorption of CsA from the gastrointestinal tract into the epithelial cells of the small intestine and (2) the transfer from the epithelial cells into the mesenteric lymph. Through our previous studies concerning the selective lymphatic delivery of drugs, we speculated that the main action of the MM system is the enhanced intestinal absorption of these drugs due to the enhanced permeability of the mucosal membrane caused by the incorporation of the lipid component of the MM system. In the studies on bleomycin, interferon and HCFU, the driving factor for the transfer of these drugs from the epithelial cells into the lymph is molecular weight. Interferon itself has a molecular weight of 23000. On the other hand, bleomycin and HCFU formed macromolecular complexes with dextran sulfate (average \( MW = 500000 \)) and \( \beta \)-cyclodextrin polymer (average \( MW = 100000 \)), respectively. However, in the case of CsA, we cannot accept our previous speculation that a certain molecular weight is required for the selective lymphatic transfer of drugs, because the molecular weight of CsA is not large, namely 1201. Thus, we must consider whether CsA itself has selective transference into the lymph rather than into the blood after being absorbed from the gastrointestinal tract. Among the materials absorbed from the gastrointestinal tract, nutritional materials such as cholesterol, long-chain fatty acids, and water-insoluble vitamins are well known to have high lymphotropic selectivity. Moreover, Palin et al. found that the highly lipophilic compounds such as 2,2, bis \( (p \)-chlorophenyl) 1,1,1-trichloroethane \( (\text{DDT}) \) and protocol having a high lymphotropic selectivity also have an affinity to chylomicron. Though the results are not shown in this paper, a CsA-MM solution was administered to two rats without the pre-administration of fresh milk. In these rats, 1 ml of saline was orally administered instead of fresh milk to maintain lymph flow. The lymph flow in these rats was about 20% decreased as compared to the rats receiving the pre-administration of fresh milk. In addition, both the lymph CsA level and the cumulative amount of CsA transferred into the thoracic duct lymph for 6 h were almost the same values as those of the rats pre-administered with fresh milk. In the case of the saline pre-administered rats, we assume that the ability of chylomicron formation was not increased. However, the lymphatic transfer of CsA was also increased by administering CsA in MM solution. Therefore, at present, we cannot deny the possibility that the selective lymphatic transport of CsA is due to the interaction of CsA with chylomicron. With respect to this point, our present results suggest that the parenteral administration route is superior to the nonparenteral route for the selective lymphatic transfer of CsA. This implies that the dissolved state of CsA in the gastrointestinal tract has an important

<table>
<thead>
<tr>
<th>Administration route</th>
<th>Cumulative amount of CsA for 6 h (( \mu g ))</th>
<th>Lymph flow (ml/h)</th>
<th>Lymph/plasma ratio</th>
<th>Number of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>19.30 ± 5.57</td>
<td>0.334 ± 0.011</td>
<td>13.26 ± 4.32</td>
<td>4</td>
</tr>
<tr>
<td>Duodenum</td>
<td>20.45 ± 12.27</td>
<td>0.292 ± 0.007(^a)</td>
<td>25.01 ± 12.65(^b)</td>
<td>4</td>
</tr>
<tr>
<td>Rectum</td>
<td>1.92 ± 0.36(^b)</td>
<td>0.361 ± 0.052</td>
<td>3.41 ± 0.95(^b)</td>
<td>4</td>
</tr>
<tr>
<td>Peritoneal Cavity</td>
<td>2.13 ± 1.50(^b)</td>
<td>0.338 ± 0.022</td>
<td>3.70 ± 0.05(^b)</td>
<td>4</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E. Statistically significant differences from intrastomach administration experiment by Student's t-test, \( a \) \( p < 0.05 \), \( b \) \( p < 0.01 \).
role in the selective lymphatic transfer of CsA. Therefore, we are now studying the effects of several solubilizers containing HCO-60 and sugar esters on the lymphatic transfer of CsA after oral administration to rats and the results will be presented in a following report.

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REFERENCES