PROMOTION OF THE SELECTIVE LYMPHATIC DELIVERY OF CYCLOSPORIN A BY LIPID-SURFACTANT MIXED MICELLES

KANJI TAKADA, NOBUHITO SHIBATA, HIROYUKI YOSHIMURA, YOSHITO MASUDA, HIROSHI YOSHIKAWA, SHOZO MURANISHI,* AND TAKAHIRO OKA**

Department of Biopharmaceutics, Kyoto Pharmaceutical University,* Misasagi, Yamashina-ku, Kyoto, 607, Japan and Second Department of Surgery, Kyoto Prefectural University of Medicine,** Hirokoji, Kamigyo-ku, Kyoto, 602, Japan

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The absorption of an immunosuppressive drug, cyclosporin A (CsA), with the aid of lipid-surfactant mixed micelles from the rat gastrointestinal (GI) tract and lymphatic delivery were studied. The administration of CsA in oily solution, sesame oil or linolic acid, into the rat duodenum indicated a small amount of CsA both in the plasma and lymph for about 6 h. The administration of CsA in the mixed micellar solution composing of linolic acid and HCO-60, polyoxyethylated (60 mol) hydrogenated castor oil, accelerated the absorption of CsA from the GI tract, and CsA was delivered into the lymphatics with an extremely high selectivity.

Keywords — cyclosporin A; immunosuppressive drug; absorption promotion; selective lymphatic delivery; rat gastrointestinal tract; lipid-surfactant mixed micelle

INTRODUCTION

Cyclosporin A (CsA), a cyclic endopeptidase, is used to inhibit the graft rejection in organ transplantation. It is extremely lipophilic and virtually water insoluble. Basic studies using rats showed that about 0.35—0.47% of the oral CsA dose is absorbed lymphatically, though the systemic availability of CsA was 21.3% in these rats. Borel et al. suggested that the immunosuppressive activity of CsA was related to a selective action against T lymphocytes which play a central role in the induction of immune responsiveness, and mainly circulate in the lymphatic system. Therefore, the immunosuppressive activity of CsA is thought to be dependent on the concentrations of the drug in the lymph.

We succeeded in the selective lymphatic delivery of drugs such as bleomycin and interferon using lipid-surfactant mixed micelles (MM) system in rats.

In this paper, we report the selective lymphatic transport of CsA by our MM system using thoracic duct cannulated rats.

MATERIALS AND METHODS

Materials — CsA was supplied by Sandoz Ltd., Basel, Switzerland. Linolic acid of 99.0% high purity grade (Nippon Oil & Fats Co., Ltd., Tokyo, Japan) and HCO-60 (Nikko Chemicals Co., Ltd., Tokyo, Japan) were used as component of MM.

Preparation of Test Solutions — Test solution of CsA in sesame oil or linolic acid was prepared by dissolving CsA in these oils. 3.5 mg/ml. MM solution was prepared by dispersing linolic acid (finally, 2.5 w/v%) containing CsA and HCO-60 (finally, 8.0 w/v%) in the distilled water followed by sonication at 25°C with an Ohtake sonicator, model 5202 (100 W, 5 min), and the resulting clear solution was used for the animal experiment. The final CsA concentration was 3.5 mg/ml MM solution.

Animal Preparation — To an anesthetized male Wistar rat weighing 350—400 g, a polyethylene cannula was surgically introduced into the left femoral artery to obtain blood samples. The thoracic duct was cannulated by a
modification of the method of Bollman et al. with a heparin filled flexible vinyl catheter and was fixed with a drop of tissue cement.\textsuperscript{81}

**Drug Administration and Collection of Blood and Lymph Samples** — After collecting blank blood and lymph samples, 1 ml of test drug solution (corresponding to a dose of CsA of 7.0 mg/kg per animal) was given into the rat duodenum. After dosing, the continuous output of lymph from the thoracic duct was collected in hourly fractions in tared culture tubes. Single blood samples (about 200 μl) were also obtained on an hourly basis in heparinized tubes and were staggered to coincide with the midpoint of the lymph collection intervals (i.e. 30, 90, 150 min, etc.). Between samplings, the femoral cannula was filled with heparinized saline to maintain its patency.

**Drug Assay** — The plasma concentration of CsA were determined with high-performance liquid chromatographic procedure previously reported in our laboratory.\textsuperscript{93} Due to a marked temperature dependence of drug partition between red cells and plasma,\textsuperscript{10} plasma was separated at 37°C from red cells immediately after the collection of rat blood. About 50—100 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 AND DISCUSSION**

As a control, CsA dissolved in sesame oil was administered into the duodenum of four rats. Lymph CsA levels reached its maximum at 2.5 h after administration, and were about 1.5 times higher than plasma CsA levels after that (Fig. 1A). When CsA dissolved in linolic acid was administered to rats, both plasma and lymph CsA levels were lower than that obtained after the administration of CsA dissolved in sesame oil (Fig. 1B). On the other hand, when CsA was administered to rats in MM solution, high concentrations of CsA were obtained in the lymph (Fig. 1C). However, only a little amount of CsA was detected in the plasma.

**FIG. 1. Plasma and Lymph Concentrations of Cyclosporin A after the Administration (A) in Sesame Oil, (B) in Linolic Acid and (C) in Linolic Acid — HCO-60 Mixed Micellar (MM) Solution into the Rat Duodenum**

○, plasma; ○, lymph. Each point represents the four individual determinations, and is expressed as the mean ± S.E.
TABLE I. Lymphatic Delivery of Cyclosporin A in Rats

<table>
<thead>
<tr>
<th>Adjuvant</th>
<th>Cumulative amount of CsA for 6 h (μg)</th>
<th>Lymph flow (ml/h)</th>
<th>Lymph/plasma ratio</th>
<th>Number of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sesame oil</td>
<td>3.34 ± 1.20 b)</td>
<td>0.508 ± 0.028 a)</td>
<td>2.71 ± 1.82 b)</td>
<td>4</td>
</tr>
<tr>
<td>MM solution</td>
<td>20.45 ± 12.27</td>
<td>0.292 ± 0.007</td>
<td>25.01 ± 12.65</td>
<td>4</td>
</tr>
<tr>
<td>Linolic acid</td>
<td>1.38 ± 0.64 b)</td>
<td>0.363 ± 0.018</td>
<td>0.84 ± 0.38 b)</td>
<td>4</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E. Statistically significant differences from MM solution experiment by Student’s t-test a) p < 0.05, b) p < 0.01.

The amount of CsA delivered into the lymph from the rat GI tract for 6 h after the administration is summarized in Table I. The administration of CsA in the MM solution showed about six-fold (to sesame oil) or fifteen-fold (to linolic acid) more amount of CsA in the lymphatics than that in the two oily dosage forms. The mean L/P ratio of CsA in the lymph to that in the plasma corresponding to the midpoint of lymph collection interval which was calculated from the data in Fig. 1 is also shown in the Table. The L/P ratio for MM solution is about ten- or thirty-fold greater than that of the sesame oil or linolic acid dosage form. The appearance of the more pronounced effect on the L/P ratio than the amount of CsA transferred into the lymph was ascribed to the fluctuation of the lymph flow between the dosage forms as reported by Palin et al.11

In our previous study concerning the oral bioavailability of water insoluble drug, rifampicin (RFP), suggested that the systemic availability of RFP dissolved in sesame oil is about two times greater than that of RFP in olive oil.12 Therefore, sesame oil was used for the standard oral CsA dosage form in this study, though the dosage form of CsA used clinically is an oily dosage form, the mixture of olive oil, pegilic 5 oleate (Labrafil® M-1994cs) and absolute ethyl alcohol (40:42:18).13 However, the amount of CsA delivered into the thoracic duct lymph for 6 h was less than 0.2% of the oral dose in sesame oil. This lymphatic delivery of CsA was extremely improved by the use of MM solution. As MM solution is composed of linolic acid and HCO-60, it is meaningful to consider the roles of linolic acid and HCO-60 on the absorption of CsA from the GI tract. It is generally recognized that linolic acid is absorbed from the GI tract and is used for the resynthesis of triglycerides, phospholipids and cholesterol esters, following transport into the lymphatics in the form of chylomicrons.14 Then, linolic acid was used as a base of MM solution. However, the lymphatic delivery of CsA was not improved from linolic acid oily dosage form. Therefore, we suppose that the important factor for the promotion of the selective lymphatic delivery of CsA by MM system exists in the dissolved state of the oil (linolic acid) in the GI tract.

We believe that this report will give a great contribution to clinicians.

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Cyclosporin A Delivery into Lymphatics


