Development of a New Carrier for Cyclosporine A With Selectivity for Lymphatics

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CYCLOSPORINE A (CyA) is a potent immunosuppressant with selective action against T lymphocytes.[1] T lymphocytes play a central role in the inhibition of immune responsiveness, and lymphocytes circulate mainly in the lymphatic systems in the body.[2] The activity of CyA is thus dependent on its concentration there. Based on this assumption, we have developed a new CyA carrier with selective lymphatic transporting efficiency, and the usefulness of this new carrier was studied from both the pharmacokinetic and pharmacologic aspects.[3-5]

METHODS

Test dosage forms are classified into four groups: (1) conventional oily (CO) solution; (2) emulsion; (3) mixed micellar (MM) solution; and (4) well-solubilized (WS) solution. Oily carrier was prepared in a 18:42:40 mixture of absolute ethyl alcohol, Nikkol TMGO-5 (Nikko Chemicals Ltd, Tokyo), and oily base, such as olive oil etc. Emulsion was prepared by dissolving CyA in oily base and by dispersing into 8% (w/v) HCO-60 solution (polyoxyethylated hydrogenated caster oil, Nikko Chemicals) followed by sonication at 25 °C for 5 minutes. MM solution was prepared by dispersing linoleic acid containing CyA and HCO-60 in distilled water followed by sonication. WS solution was prepared by dissolving CyA in 8% (w/v) HCO-60 or 2.0% (w/v) sugar ester solution (DK ester, San-ei Chemicals Ltd, Toyonaka) followed by sonication. The final CyA concentration of these test solutions was 3.5 mg/mL.

The effect of administration route on the selective lymphatic delivery of CyA was studied using male Wistar rats in which the thoracic lymph duct was cannulated with plastic tubing. After collection of normal lymph, MM solution was administered, 7 mg/kg, by four administration routes, intrastomach (IS), intraduodenum (ID), intraperitoneal (IP) and rectally, and lymph and blood samples were collected for six hours. By centrifuging the blood samples at 37 °C, plasma samples were obtained. CyA content in lymph and plasma samples was measured by a high-performance liquid chromatographic (HPLC) method developed here.[6]

Lymphatic availability study was also performed in rats with the test solutions at dose levels of 7 mg/kg, and the lymphatic CyA levels were measured.

Heterotopic cardiac transplantation was performed by intrarenal anastomosis of donor aorta and pulmonary artery to recipient aorta and inferior vena cava, respectively. Buffalo rats provided by Dr Aizawa (Hokkaido University) were donors and Wistar rats obtained from Shionagi Pharmaceutical Co Ltd were recipients of heart allografts. Allograft recipients were given CO solution or 8% HCO-60 solution of CyA at dose levels of 1 and 2 mg/kg/d for 1 week. Rejection was defined as cessation of palpable heart beat and was confirmed by direct inspection at laparotomy and histologic examination.

Pharmacokinetic study was performed in three groups of rats. One group received CO solution; the second group received 8% HCO-60 solution at an oral dose level of 7 mg/kg; the third group received IV CyA dose, 3.5 mg/kg. Blood samples were collected for eight hours from the carotid artery through a cannula, and all of the gastrointestinal (GI) tract was removed at the end of the experiment. CyA contents in plasma, GI tract, and GI contents were measured by HPLC method. The area under the plasma CyA concentration *v* time curve (AUC) was calculated by a trapezoidal rule. All values are represented as the mean ±SE.

RESULTS

The rectal or IP administration of CyA in MM solution showed small amounts of CyA in the lymph for six hours. However, oral administration gave high lymph CyA concentrations. IS administration of CyA resulted in the highest CyA levels in lymph, 16 μg/mL, about twenty times higher than those obtained after rectal or IP administration. The lymphatic availability of CyA was affected by its dosage. As the solubilization of CyA was accelerated, the amount of CyA transferred

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0041-1345/87/1901-0634$03.00/0

into the thoracic duct lymph was increased. The rank order of lymphatic availability was WS solution > MM solution > emulsion > CO solution. The percentage of CyA transferred into the lymph for six hours from 8% HCO-60 solution was 2.14 ± 0.04%, and the maximum lymph CyA level was 57.10 ± 13.33 μg/mL. DK ester solution also showed high values, namely 1.62 ± 0.92% and 46.35 ± 10.43 μg/mL. On the other hand, less than 0.2% of the administered CyA dose was transferred into the lymph from the CO solution.

The immunosuppressive activity of CyA was increased by oral administration of CyA in 8% HCO-60 solution. The mean survival of heart allograft in rats treated with daily CyA doses in 8% HCO-60 solution, 2 mg/kg, for 1 week, was 21.8 ± 10.5 (SD) day, and two rat hearts survived more than 40 days. The mean survival time from the CO solution, 2 mg/kg/d for 1 week, was 12.8 ± 1.9 (SD) day. The control (nontreated rats) mean survival day was 7.6 ± 0.6 (SD) day. In the pharmacokinetic study, the percentage recovery of CyA from the GI tract after oral administration of the CO solution was 44.5 ± 1.36%, whereas 29.1 ± 3.07% was obtained after administration in 8% HCO-60 solution. The systemic availability of CyA, measured as the ratio, AUC(oral)/AUC(iv), was 28.71% for CO solution and 54.60% for HCO-60 solution.

DISCUSSION

HCO-60 is a well known nonionic surfactant and is used as a pharmaceutical additive.[7] We have been using HCO-60 as an absorption promoter to increase the systemic availability of percutaneously administered drugs,[8] or antitumor drugs[9] and to increase the lymphatic availability of interferon.[10] In this experiment, both the lymphatic and systemic availability of CyA was increased by HCO-60. However, the improved lymphatic availability of CyA was less than 5% of the oral dose, and the contribution of the amount of CyA delivered through the lymphatic route to the systemic circulation was very small. On the other hand, the immunosuppressive activity of CyA was significantly increased by this new career, 8% HCO-60 solution. The precise mechanism of this intensified immunosuppressive activity of CyA is now under investigation.

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