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Implantable slow release cyclosporin A (CYA) delivery system to thoracic lymph duct

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Abstract

An implant from which cyclosporin A (CyA) is slowly released has been prepared and the immunosuppressive effect of such a CyA implant was studied with an allogenic rat heart transplantation model system. The implant was made of a biodegradable polymer of L-lactic acid (PLA) and/or co-polymer of L-lactic acid and glycolic acid (PLGA) as the base. The synthetic nonionic surfactant, HCO-60 (polyoxyethylated, 60 μmol, castor oil derivative), was used as an additive. By changing the contents of the polymer and HCO-60, the release rate of CyA was adjusted. To determine the kind and formulation of the base, the lipophilic dye, Sudan Black (SB), was used as a model drug for CyA. Implants made of PLGA and HCO-60 (10:15) released more than 45% of SB within 6 days, whereas PLA implants released less than 15%. Based on these results, an implant containing CyA from which $70 \pm 9.7\%$ of the formulated CyA was released within 6 days was prepared. The size of the implant was 2 mm (o.d.) $\times$ 15 mm length. The immunosuppressive activities of CyA administered as an implant attached to the thoracic duct were evaluated with an allogenic rat heart transplantation model system. As a control, a placebo implant was used. The CyA implant showed a cardiac graft survival period of 9.8 ± 1.3 (mean $\pm$ S.D.) and 21.0 ± 9.6 days at 20 and 35 mg/kg, respectively, which is significantly longer than that of the placebo implant rat group (7.6 ± 0.9 days). These results demonstrate the possibility of local immunosuppression at the thoracic lymph in immunosuppressive therapy with CyA.

Keywords: Cyclosporin A; Implant; Lymphatic system; Thoracic duct; Local immunosuppressive therapy; L-Lactic acid; Glycolic acid; Rat

1. Introduction

In the field of organ transplantation, cyclosporin A (CyA) is a widely used immunosuppressant (Cohen et al., 1984). CyA is known to inhibit T cell cytokine gene transcription (Kronke et al., 1984). Therefore, the target cell of CyA among lymphocytes is T cells (Baumann et al., 1992). In the body, lymphocytes have the property of returning to their homes from the central circulation (O'Driscoll, 1992). During the homing process, the passage of lymphocytes through the lymphatic system occurs with high frequency.

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Namely, the lymphocyte density in the lymphatic system is extremely greater than that in the systemic circulation. By assuming that the pharmacological activity of CyA is related to its concentration in the lymphatic system where lymphocytes exist in large quantities, we have been studying an immunosuppressant delivery system that provides CyA transfer into the lymphatics. Namely, an enteric solid dispersion system of CyA as an oral CyA lymphatic delivery system has been reported (Takada et al., 1985, 1986a,b, 1987, 1988, 1989a,b). On the other hand, intravenous (i.v.) CyA therapy is clinically performed on the day of transplantation and thereafter for several days. The i.v. CyA solution is an oily solution provided by Sandoz Co. Ltd. Using this i.v. solution, lymphatic transport of CyA was investigated. The percentage of CyA transported into the thoracic lymph was 0.25% (Ueda et al., 1983). However, clinical CyA use in currently accepted dosage schedules promotes the development of infection, diabetes and hypertension in allograft recipients, and is associated with an increased risk of malignancy. Several approaches have been proposed toward reducing the drug-specific and general adverse consequences of systemic immunosuppression in allograft recipients. In particular, utilization of a local drug administration system causing local immunosuppression has attracted our interest. Anatomically, lymph fluids are recirculated into the central circulation (O'Driscoll, 1992). Especially, before entering into the blood circulation, the thoracic duct collects the lymphatic fluid from the other parts of the body. Therefore, there is a possibility of achieving local immunosuppression by administering immunosuppressants into the thoracic duct. As the lymph duct is very erodible, the penetration of CyA into the thoracic duct is thought to occur with ease. However, our previous report suggested that the penetration of CyA into the thoracic duct after application onto the surface of the thoracic duct is less than that obtained after i.v. injection (Katayama et al., 1994). Therefore, an implant from which CyA is slowly released has been prepared. In this report, the local immunosuppression by a CyA implant has been evaluated in a rat allogenic heart transplantation model.

2. Experimental

2.1. Materials

CyA (cyclosporin A) powder was kindly supplied by Sandoz Ltd, Basel, Switzerland. Sandimmun®, i.v. oily solution, in which the CyA concentration is 100 mg/ml was obtained from Japan Sandoz Ltd (Tokyo, Japan). Sudan Black B (SB) was obtained from Tokyo Chemicals Industry Ltd (Tokyo, Japan). A synthetic nonionic surfactant, HCO-60® (polyoxyethylated, 60 μmol, castor oil derivative), and polysorbate 80 (Tween 80) were obtained from Nikko Chemicals Co., Ltd (Tokyo, Japan) and Nacarai Tesque, Inc. (Kyoto, Japan), respectively. Polymer of L-lactic acid (PLA) and co-polymer of both L-lactic acid and glycolic acid (PLGA) was obtained from Mitsui Toatsu Chemicals, Inc. (Tokyo, Japan). All other reagents were commercial products of reagent grade. Inbred Buffalo rats (RT-1b) were obtained from Clea Japan Inc. (Tokyo, Japan). Inbred Lewis rats (RT-11) were obtained from Oriental Yeast Co., Ltd (Tokyo, Japan).

2.2. Preparation of implants

Three kinds of implants containing SB were prepared with PLA and/or PLGA as the base. The chemical compositions were polymer/SB/HCO-60 = 90 : 2 : 5, polymer/SB/HCO-60 = 30 : 2 : 10, polymer/SB/HCO-60 = 10 : 2 : 15, respectively. For example, for the preparation of the first implant, 450 mg of PLA, 10 mg of SB and 25 mg of HCO-60 were weighed and then used for the preparation. The composition of an implant containing CyA was PLGA/CyA/HCO-60 = 10 : 2 : 15. To the mixture of these compounds, 2 ml of methylene chloride was added and dissolved thoroughly. Stirring was continued at room temperature (23°C) until about 3/4 of the solvent disappeared. The resulting semi-solid mixture was poured into a Teflon mold (2 mm × 15 mm). After thorough drying in a desiccator overnight at room temperature, the implant was isolated from the mold. After measuring the body weight of the recipient rat, the amount of CyA contained in the implant was adjusted to a dose of 15 mg/kg rat body weight.

2.3. In vitro release experiment

The study of SB and CyA release from the individual preparations was performed according to the our previously described method (Takada et al., 1989a). The test fluid employed was isotonic pH 7.4 phosphate buffer containing Tween 80 (0.6% w/v). A sample of implant containing about 3 mg of SB or CyA was put into a vinyl cage (4 cm × 3 cm). The cage was put into 100 ml of the test fluid maintained at 37°C. The test fluid was rotated at 150 rpm with a stirrer bar (5 mm × 20 mm). 5-ml aliquots of the sample were taken at 0, 1, 2, 3, 4, 5 and 6 days after the start of the experiment. The drug content was determined by a visible or UV absorption method as described in the following section. After measuring the absorbance, the sample was returned to the dissolution medium. All release experiments were carried out in triplicate.

2.4. Allogenic rat heart transplantation

Allogenic rat heart transplantation was performed using a cuff technique developed by Heron (1971) and Matsuura et al. (1991). Male Lewis rats (300–350 g) and male Buffalo rats (200–250 g) were used as recipients and donors, respectively. The rats were anesthetized with sodium pentobarbital (45 mg/kg). To remove the donor heart, a midline abdominal incision was made in the donor animal, and 2–3 ml of Krebs-Henseleit solution containing 50 units of heparin was injected into the femoral vein. Ligatures were performed around the superior and inferior vena cavae. The latter ligature was knotted following infusion of 10 ml Krebs-Henseleit solution through the thoratic aorta into the heart. Thereafter, the pulmonary artery and the thoracic aorta were transsected 3–5 mm distal to their origin. The heart was removed and was placed in ice-cold Krebs-Henseleit solution. Next, a vertical incision was made into the neck of recipient rat. CyA i.v. solution was injected into the rat (5 mg/kg) through the jugular vein just before the heart transplantation. The external jugular vein and the common carotid artery were dissected. The cuffs consisted of a cuff body 3.0 mm in length and a 1.0 mm cuff extension made of a Teflon i.v. cannula (24 gauge, outside diameter of 0.60 mm) for the carotid artery and (22 gauge, outside diameter of 0.80 mm) for the external jugular vein, respectively. The donor heart was transferred to the neck of the recipient. The pulmonary artery was drawn over the jugular vein cuff. The thoracic aorta was also anastomosed to the carotid artery. The incision was sutured. Subsequently, abdominal incision was performed and the implant preparation was inserted along with the thoracic duct. The insertion site was covered with Novix® film (Iwaki Glass Co., Tokyo) in order to avoid the absorption of CyA from the other site except the thoracic duct. After the abdominal incision was sutured, the recipient rat was maintained in the cage. The survival of the tranplanted heart was monitored every day by measuring the transplanted heart beat. When a heart beat was not detected, the rat was killed and the heart was isolated. Graft heart was placed in buffered formalin for 24 h, then serially sectioned in wax blocks and stained with hematoxylin and eosin for histologic examination under light microscopy.

2.5. Drug assay in dissolution experiment

The concentration of SB or CyA in the dissolution test fluid was performed by determining its absorbance at 660 and 210 nm, respectively. The visible and UV absorbance of the sample was detected using a Shimadzu UV-Visible recording spectrophotometer model UV-240 (Kyoto, Japan). The standard curve of SB or CyA was linear over the range of 5–50 μg/ml.

2.6. Statistical analysis of the data

Wilcoxon's rank test was used to assess the significance of differences among animals in each group.

3. Results

As there are no reports concerning the release characteristics of highly lipophilic drugs from the

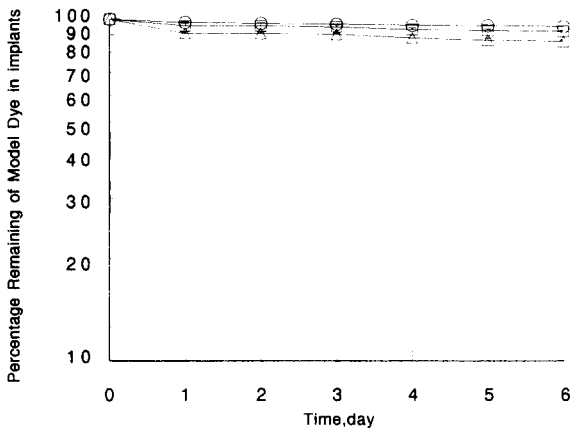

Fig. 1. Percentages remaining of SB in implants after the start of the release experiment. (○) Implant composed of PLA/SB/HCO-60 = 90:2:5; (□) implant composed of PLA/SB/HCO-60 = 30:2:10; (△) implant composed of PLA/SB/HCO-60 = 10:2:15.

PLA or PLGA implant, a release study was performed using Sudan Black (SB) as a model of a highly lipophilic drug such as cyclosporin A. Three kinds of SB implants in which PLA was used as the base were prepared at first and the release characteristics of SB from the implants were assessed in the in vitro dissolution experiment. An implant containing 3.0 mg of SB was used in each experiment and the dissolution behavior of SB from the implants was monitored for 6 days. As shown in Fig. 1, the release behavior of SB was dependent on the formulation. Although the data are not shown in Fig. 1, an implant which does not contain HCO-60, of which the chemical composition was PLA/SB = 30:2, was prepared and the release test was also performed. However, the marker drug (SB) was not released until the end of the experiment. To accelerate the release rate of SB, HCO-60 was formulated into the implant. Therefore, three kinds of implants containing PLA, SB, and HCO-60 were prepared and the dissolution behavior of SB was studied. As shown by Fig. 1, the release rate of SB increased with increasing content of HCO-60 in the implant. However, even in the case of the implant containing the greatest amount of HCO-60 (PLA/SB/HCO-60 = 10:2:15), the percent amount of SB released at the end of the experiment was 13.5 ± 0.9% as shown in Table 1. By the addition of HCO-60 into the implant, the amount of SB released over the 6 day period increased from 4.2% to 7.4 and 13.5%, respectively.

Table 1
Percent release of Sudan Black (SB) and cyclosporin A (CyA) from implants in dissolution experiment

Composition of implant	% released over 6 days
PLA/SB/HCO-60 = 90:2:5	4.2 ± 0.3
PLA/SB/HCO-60 = 30:2:10	7.4 ± 0.5
PLA/SB/HCO-60 = 10:2:15	13.5 ± 0.9
PLGA/SB/HCO-60 = 90:2:5	4.1 ± 1.9
PLGA/SB/HCO-60 = 30:2:10	9.2 ± 2.0
PLGA/SB/HCO-60 = 10:2:15	46.3 ± 3.2
PLGA/CyA/HCO-60 = 10:2:15	70.0 ± 9.7

All data are the mean ± SD.

On the other hand, implants in which the co-polymer, PLGA, was used as the base were prepared and the effect of HCO-60 on the release rate of SB was also examined. Fig. 2 shows the results. For two implants containing less HCO-60, the percent amount of SB remaining in the implants was 95.9 ± 2.4 and 90.8 ± 2.4% at the end of the experiment, respectively. However, in the case of the implant in which the amount of HCO-60 increased (PLGA/SB/HCO-60 =

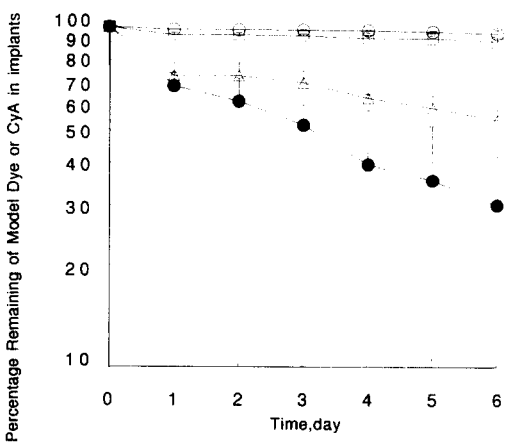

Fig. 2. Percentages remaining of SB and CyA in implants after the start of the release experiment. (○) Implant composed of PLGA/SB/HCO-60 = 90:2:5; (□) implant composed of PLGA/SB/HCO-60 = 30:2:10; (△) implant composed of PLGA/SB/HCO-60 = 10:2:15; (●) implant composed of PLGA/CyA/HCO-60 = 10:2:15.

10:2:15), the release rate of SB increased considerably. Although a clear burst phenomenon was observed during the early period of the experiment, about 46% of SB was released within 6 days. By comparing the percent released of the corresponding PLA implant, the percent released of this PLGA implant increased about 3-fold, from 13.5 to 46.3%. Based on these results, an implant containing CyA was prepared using PLGA as the base, in which the chemical composition of three components was PLGA/CyA/HCO-60 = 10:2:15. The release behavior of CyA was confirmed by carrying out the same release experiment and the results are also depicted in Fig. 2. It was confirmed that CyA is released more rapidly than the other two kinds of SB implants. Until the end of the study, 6 days, almost 70 ± 9.7% of CyA had been released from the implant.

To study the pharmacological efficiencies of the thus obtained implants, an allogenic rat heart tranplantation model was used. As test preparations, not only a placebo implant containing PLGA and HCO-60, PLGA/HCO-60 = 10:15, but also an implant composed of PLGA/CyA/HCO-60 = 10:2:15 were used. At day 0 when the rat received a tranplanted heart, a CyA implant containing 15.0 or 30.0 mg of CyA/kg body weight was applied to the abdominal cavity just contacting the thoracic duct. In addition, just before transplantation, CyA solution (5 mg/kg) was injected through the jugular vein for the sake of suppression of the initial acute rejective reaction against the transplanted heart. After day 0, no CyA was administered to the recipient rats. The survival of the transplanted heart was monitored based on the heart beat. Table 2 summarizes the results of each group. In the control group rats receiving placebo implant (group 1), the mean survival time (MST) of the transplanted heart was 6.4 ± 1.9 days. This result is consistent with our previous observation where the recipient rats received oral placebo tablets (Yasumura et al., 1986). Group 2 rats which received a single i.v. dose of CyA (5 mg/kg) and placebo implant rejected the hearts with an MST of 7.6 ± 0.9 days. Group 3 rats receiving CyA implant at a dosage of 15 mg/kg on day 0 survived significantly longer (MST 9.8 days) than the control group ($P < 0.01$). Group 4 rats were treated with the same formulation implants with twice the CyA dose (30 mg/kg). In this group of rats, the MST was prolonged considerably as compared to rats of group 3. In particular, in rats of group 4, the transplanted hearts survived more than 20 days (MST 21.0 ± 9.6 days). These results demonstrate the efficiency of CyA implant in rat heart transplantation.

Table 2
Pharmacological effect of implants on the cardiac allograft survival time in rats

Rat group	Dose of CyA (mg/kg)	Survival time (days)	MST [a] (±SD) (days)
1	–	4, 5, 7, 7, 9	6.4 ± 1.9
2	5.0	7, 7, 7, 8, 9	7.6 ± 0.9
3	20.0 (5.0 + 15.0)	9, 9, 9, 10, 12	9.8 ± 1.3
4	35.0 (5.0 + 30.0)	10, 16, 21, 22, 36	21.0 ± 9.6

Group 1, administration of placebo implant; group 2, administration of both CyA i.v. solution (5 mg/kg) and placebo implant; groups 3 and 4, administration of both CyA i.v. solution and CyA implant. All data are the mean ± SD.
[a] Mean survival time.

4. Discussion

Recent advances in organ transplantation require immunosuppressive therapy. Among immunosuppressants, CyA is a valuable and potent immunosuppressant in transplantation immunology (Borel et al., 1989; Thomson, 1989). CyA increases the survival of transplants by inhibiting the T lymphocyte compartment responsible for rejection (Sigal et al., 1992; Colvin et al., 1993). Although CyA displays an amazingly low immunosuppressive hazard and offers a secure path on the 'slippery slope' of therapy, pleiotropic, nonimmunologic, vasculopathic side effects of CyA preclude the use of sufficient doses to fully elucidate its activity in immunosuppressive tharapy. Clinically important side effects of CyA are nephrocyto- and hepatocytotoxicities and central nervous toxicity (Awni, 1992). To avoid such side effects and to elicit the immunosuppressive activity of CyA safely, local immunosuppression is

a valuable approach. As lymphocytes circulate through the thoracic duct with high density, we propose that local immunosuppression would be possible by delivering more CyA to the thoracic duct. To increase the CyA concentration in the thoracic duct, the direct administration of CyA into the thoracic duct is the most effective method. However, such a direct administration method is not applicable in clinical immunosuppressive therapy. Our implant system for CyA is one of the probable methods to deliver CyA into the thoracic duct. In this experiment, the survival of the heart graft in active implant groups has been significantly prolonged as compared to the placebo implant group. However, in our previous report, 12.8 ± 1.9 days was obtained as the MST in the heart-transplant rats who received CyA at 2 mg/kg per day for 7 consecutive days. In that case, the total CyA dose was 14.0 mg/kg. Therefore, from the pharmacological point of view, it is difficult to support the superiority of implant systems over conventional immunosuppressive therapy. In addition, our previous report suggested that both the amount of CyA transported into the thoracic duct and the maximum transport rate cannot be improved by the direct administration of CyA onto the thoracic duct as compared to i.v. CyA administration (Katayama et al., 1994). In this study, as the administered CyA dose was 20 or 35 mg/kg, slow release characteristics were conferred on the implant. Therefore, even if a pharmacokinetic study were to be performed in these recipient rats, the resulting plasma CyA levels would be below the detection limit of our HPLC assay method. Therefore, the plasma CyA concentration was not measured in this experiment. According to the reason described above, biopharmaceutical evaluation of the implant could not be performed. Instead, evaluation of the CyA implant system has been performed by a pharmacological experiment using a rat allogenic heart transplantation system. As an experimental rat heart transplantation method, transplantation of a donor heart to the abdominal aorta and inferior vena cava in the abdominal cavity by anastomosis are involved (Yasumura et al., 1986). However, this method necessitates extensive training in microsurgery. In addition, it is more difficult to monitor the transplanted heart beat as compared with the cuff method used in this experiment, as the transplanted heart is localized deep within the abdominal cavity. This study has overcome these problems. By means of the cuff method, the pharmacological efficiency of the new CyA dosage form has been confirmed.

In conclusion, as a means of local immunosuppression, an implant from which CyA is released slowly has been prepared and the pharmacological efficiency of the CyA implant has been evaluated by performing an allograft rat heart transplantation experiment. The CyA implant was administered onto the surface of the thoracic duct. Prolongation of the heart graft has been observed as compared to the control experiment in rats which received placebo implants. However, from the standpoint of pharmacological evaluation, it was difficult to ascertain the superiority of the implant system over the conventional consecutive administration therapy of CyA. The implant system has the advantage that the recipient may be spared from daily CyA administration.

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