LYMPHOTROPIC DELIVERY OF CYCLOSPORIN A BY INTRAMUSCULAR INJECTION OF BIODEGRADABLE MICROSPHERES IN MICE

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Biodegradable microspheres containing cyclosporin A (CsA), an immunosuppressant, enabled the lymphotropic delivery of the drug over the long term by parenteral administration in mice. The CsA encapsulated in the microspheres of polylactic acid polymer (Mw, 9000) was released over 30 days in vitro. The intramuscular injection of the microspheres containing CsA in the femoral site of mice maintained the drug at a high level in the inguinal lymph nodes over 1 month, although only low blood levels of the drug were observed.

KEY WORDS cyclosporin A; lymphatic delivery; microsphere; lymph node; intramuscular injection

Cyclosporin A (CsA) is currently the main immunosuppressive agent which has resulted in considerable improvement in graft survival in various organ transplantations. It is reported that the immunosuppressive activity of CsA is related to a selective inhibitive action against T lymphocytes, which play a core role in the induction of immune responsiveness. These lymphocytes circulate mainly in the lymphatic system. On the other hand, CsA sometimes causes side effects, the degree of which is thought to be dependent on the systemic blood level of CsA. These facts suggest that increasing the lymphatic level of CsA as well as reducing its blood concentration would enhance the therapeutic index of the drug. Regarding this point, Takada et al. found that some solubilizers selectively increase the lymphatic level of CsA from the enteral route which enhanced the immunosuppressive effect of the drug in an animal transplant model. Several investigators have reported on the availability of topical dosing of CsA using a controlled-release system with the aid of particulates or microspheres. However, there has been no attempt to deliver CsA into the lymphatic system selectively under controlled release. In this paper, we describe the specific delivery of CsA to the lymph nodes in mice with controlled release, which was enabled by the intramuscular administration of biodegradable microspheres containing the drug.

MATERIALS AND METHODS

CsA was supplied by Sandoz Ltd., Switzerland and [3H]CsA was purchased from Amersham Life Science, UK. L-polyactic acid with average molecular weight of approximately 9000 calculated from the intrinsic viscosities was from Makro Tek., Germany. All other chemicals were of reagent grade and obtained commercially.

Microspheres of polylactic acid containing labeled CsA were prepared by a solvent-evaporation method. Two mg of unlabeled CsA and 18 mg of polylactic acid were dissolved in 2 ml of methylene chloride. One ml of [3H]CsA (1 mCi/ml) was added to this solution and the mixture was poured into 7 ml of 2% (w/v) polyvinylalcohol aqueous solution followed by sonication at 100 W. The resulting emulsion was stirred at 500 rpm with a magnetic stirrer for 20 h at room temperature to evaporate off the methylene chloride. The microspheres were collected by centrifugation and dried after several washings with distilled water.

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In vitro release of CsA from microspheres was done as follows. Ten mg of microspheres containing different amounts of unlabeled CsA alone was suspended in a glass tube filled with 50 ml of saline solution containing 0.01% (w/v) polysorbate 80. The tube was immersed in a water bath shaker at 37°C and shaken horizontally. At periodic intervals, medium was removed for drug analysis and the same amount of fresh medium was added to the tube. The amount of CsA released was measured by a specific monoclonal RIA method using Cyclo-Trac SP® (Inestar Co., U.S.A.).

In vivo administration of CsA was carried out using CD1 male mice. The CsA solution (1 mg/ml) was prepared by dissolving unlabeled CsA in ethanol which was added to same volume of [³H]CsA. Microspheres containing CsA (10% w/w) were dispersed in saline solution 10 mg/ml. Groups of five mice that were anesthetized intraperitoneally with pentobarbital, body weight 20 g, were injected intramuscularly into the femoral site of the right leg with either CsA solution or a suspension of CsA loaded in microspheres (dose of CsA: 1 mg/kg). Mice were killed at various time points after anesthesia and the inguinal lymph nodes were extirpated. Blood samples were also taken by cardiac puncture just before killing. All mice procedures were carried out in accordance with the "Principles of Laboratory Animals Care" published by the NIH.

Plasma levels of drug were determined by measuring the tritium level with the aid of liquid scintillation counting after adding a scintillation cocktail. The lymph nodes were homogenized, and methylene chloride added and centrifuged followed by 20-min shaking. These samples were also analyzed by scintillation counting.

RESULTS AND DISCUSSION

CsA was entrapped with high yield (88-93% recovery) in microspheres due to the high lipophilicity of CsA and microsphere materials. Observation of microspheres with a scanning electron microscope showed that the mean diameter of the microspheres was approximately 260 nm. Figure 1 indicates the release patterns of CsA in saline solution from microspheres having drug contents of 5.5%, 10.2% and 15.8% (w/w), respectively. All the preparations showed a release rate of the drug which was low after the burst during the initial several days. The drug-release profiles of the three preparations were almost the same, with only the difference being in the amount released owing to the drug level loaded in the microspheres. The calculated percentages of amounts of CsA released at 30 days from the three preparations ranged from 36% to 39%.

Figure 2 shows the CsA levels in plasma and inguinal
lymph nodes from the footpad after intramuscular injection of CsA solution. The CsA level reached a maximum at 2 h and 4 h after administration in the plasma and lymph nodes, respectively. Although CsA levels in the lymph node were higher than those in plasma, no significant difference was observed. CsA levels in plasma and lymph nodes were diminished under the limit of detection at 24 h and 36 h after injection, respectively. Little is known about the transport of CsA into the lymph nodes from parenteral route, but as can be observed in Figure 2, CsA itself does not possess the lymphotropic properties.

The inguinal lymph node concentration and plasma level of CsA after injection of CsA-loaded microspheres are indicated in Figure 3. The level of CsA in the lymph nodes reached a peak 2 days after injection. By injection with microspheres, lymph node levels of the drug were greatly increased (approximately 20 fold at peak time) over the concentrations obtained with solutions, as shown in Figure 2. Then the lymph node level of CsA decreased gradually, and even at 30 days after injection a sufficient level of the drug was observed. On the other hand, following the injection of CsA microspheres, all the plasma concentrations were observed to be under the detectable levels during the period of 1 to 30 days after administration. This finding confirms that lymphotropic delivery of CsA was possible due to the encapsulation of the drug in microspheres.

Transport of chemicals into the circulatory system from tissue is dependent on their permeability through the capillary vessels. The permeability of blood capillaries is much less than that of the lymphatic vessel wall, which has large pores and clefts. Therefore, we consider that the selective lymphatic delivery of CsA by the microspheres in this study is dependent on the size-sieving mechanism through the capillary wall.

The polylactic acid used here has excellent biodegradable and biocompatible properties and is degraded to water and bicarbonate through the monomer of lactic acid in the body. Accordingly, microspheres composed of polylactic acid should be suitable drug carriers. Total concentrations of CsA were measured here, and we will distinguish the lymphatic level of free CsA from that of entrapped drug in the near future. This will more precisely elucidate the usefulness of the lymphotropic microsphere system to enhance the therapeutic effect of CsA. To our knowledge, this is the first report demonstrating the potential of microspheres for selectively delivering CsA to the lymph nodes over the long term by parenteral administration.

REFERENCES


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