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Sustained Release of Insulin from a Hydrophilic Polymer Matrix Implanted in Diabetic Rats¹⁾

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A long-acting insulin preparation involving the sustained release of biologically active insulin from a hydrophilic ethylene-vinyl alcohol (EVAL) matrix was developed. A single subcutaneous implant of this insulin lowered the blood glucose levels of diabetic rats for more than one month. These results suggest that a long-acting insulin may be produced by the entrapment of insulin within a hydrophilic EVAL copolymer matrix.

Keywords—ethylene-vinyl alcohol copolymer; insulin; drug delivery; implant; sustained release; matrix system; diabetic rat

Many biomedical polymers have been utilized to develop implantable drug delivery systems. Sustained-release systems that use implanted polymeric devices can deliver a steady quantity of drugs to a target area over long periods of time. We have reported that matrices composed of ethylene-vinyl alcohol (EVAL) copolymer could be useful vehicles for implanted delivery systems for anticancer agents.²⁻⁵⁾ The unique characteristic of this copolymer is its hydrophilicity.³⁾ The EVAL copolymer may be applicable as a unique drug carrier for implanted devices because of its hydrophilic character.

Attempts have been made to prolong the action of insulin by implanting insulin-containing polymer under the skin of animals.⁶⁻⁸⁾ A single subcutaneous implant of an insulin-containing polymer (for example, ethylene-vinyl acetate; EVAc) provided up to one month of sustained release of insulin in diabetic rats.⁷⁾

In this work, as a part of a series of studies on pharmaceutical applications of EVAL copolymers, EVAL admixed with insulin was been subcutaneously implanted in diabetic rats and the performance of these animals with respect to blood glucose levels and growth was evaluated.

Experimental

Materials—Bovine insulin (24 I.U. per mg) was obtained from Sigma Chemical Co., St. Louis. EVAL copolymers with 32 mol% of ethylene unit were gifts from Kuraray Co., Tokyo.

Preparation of Insulin-EVAL Matrix—Sustained-release EVAL copolymer matrices containing insulin were prepared based on the method of Miyazaki *et al.*³⁾ with slight modifications. They were fabricated to be 1.6 cm in diameter, 0.1 cm in thickness, and 0.27 g in weight. The EVAL copolymer was dissolved in the solvent (*n*-propyl alcohol: water = 3:1) at 80–85°C. After cooling of the EVAL solution to room temperature, bovine insulin was mixed into it. The resulting suspension was poured into a plastic mold and kept at room temperature for 1 h. Then, disks were cut from the polymer-insulin mixture and dried for 2 d at room temperature *in vacuo*. The resulting disks were placed in 10 ml of phosphate buffer (pH 7.0) for 1 h to hydrate them, and then implanted subcutaneously in the lower abdomen as described below. Insulin that might have adhered to their surface could diffuse away.

Animals—Male Wistar rats weighing 170–185 g were used. Each animal was made diabetic by an intravenous injection of freshly prepared alloxan solution (70 mg/kg). The rats were left untreated for 5 d and were then entered into the experimental design.

Implantation of EVAL Matrices—The polymer implants were made by using a modification of our previously described procedure.⁵⁾ The rats were anesthetized with pentobarbital and the insulin-EVAL copolymer matrix was implanted subcutaneously in the lower abdomen of the animals, by means of a small incision on the skin. The skin incision was closed with adhesive for medical use. To measure glucose levels, 100 μ l of blood was withdrawn from the tail and centrifuged at 3000 rpm. Thirty-microliter samples of plasma were analyzed by the glucose-oxidase/peroxidase method.⁹⁾

Results and Discussion

The blood-glucose levels of diabetic rats implanted subcutaneously with 9.8 mg of insulin-EVAL matrices are shown in Figs. 1 and 2. After implantation of the EVAL matrices, blood glucose levels gradually decreased from 716.2 to 94.6 mg/dl in 6 h (Fig. 1) and then remained stable for at least 30 d (Fig. 2). During the 30-d experiment, the mean glucose level for treated animals was 180.1 mg/dl. In contrast, all control animals receiving empty EVAL matrices (*i.e.*, without insulin) displayed blood glucose levels between 462.3 and 740.5 mg/dl throughout the experiment. These results clearly show that insulin in EVAL copolymer implants can release insulin in biologically active form for a period of at least one month.

In a preliminary *in vitro* experiment on polymer design, approximately 10% of the total drug was released in 6 h, indicating that this polymer device would be capable of releasing drugs for longer periods of time.³⁻⁵⁾

The mechanism of release of bioactive macromolecules such as insulin from EVAL matrices is not well understood. The EVAL copolymer is hydrophilic in nature, and the matrices are capable of imbibing adequate quantities of water, through which the dissolved

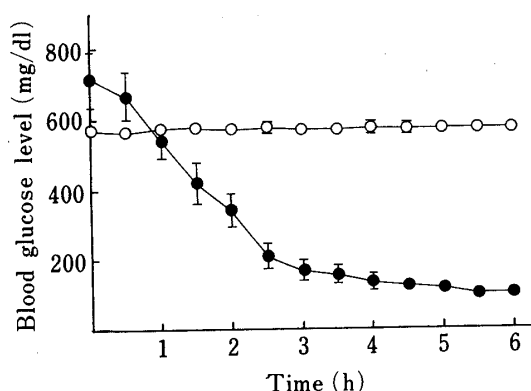


Fig. 1. Blood Glucose Levels of Diabetic Rats Implanted with a Single Dose of EVAL Copolymer Matrices with (●) and without (○) 9.8 mg of Insulin for 6 h

Each value is the mean \pm S.E. of 3–4 experiments.

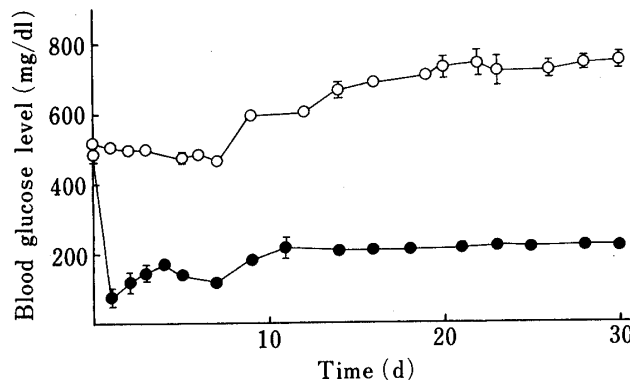


Fig. 2. Blood Glucose Levels of Diabetic Rats Implanted with a Single Dose of EVAL Copolymer Matrices with (●) and without (○) 9.8 mg of Insulin for 30 d

Each value is the mean \pm S.E. of 3 experiments.

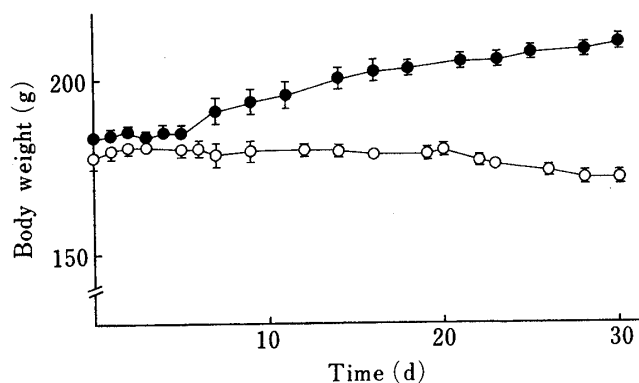


Fig. 3. Weight Changes in Diabetic Rats Implanted with a Single Dose of EVAL Copolymer Matrices with (●) and without (○) 9.8 mg of Insulin

Each value is the mean \pm S.E. of 3 experiments.

insulin might diffuse.

Figure 3 shows the change in body weights in experimental animals implanted with EVAL matrices. Animals with insulin-EVAL matrices started with body weights of approximately 183.3 g at day 0, and the mean body weights at day 30 had risen to 210.7 g. In contrast, diabetic controls receiving matrices without insulin lost weight and at the end of the experiment their mean body weight was 171.3 g. The weight gain in the controls was depressed as a result of the disease.

The subcutaneous implantation of a single dose of insulin-EVAL matrices in diabetic animals provided a sustained release of insulin for more than one month as demonstrated by the lowering of blood glucose levels and the increase in body weights. The subcutaneous implantation of empty EVAL matrices in diabetic rats had no effect on the animal's blood glucose levels or body weight.

Commercially available insulin suffer from the disadvantage of having a short half-life *in vivo*. The effective half-life of insulin has been increased substantially by the use of insulin-containing EVAL polymer matrices. The EVAL copolymer prepared from EVAc copolymer is nontoxic, flexible, and heat-processable. The unique characteristic of this copolymer, different from EVAc copolymer, is its hydrophilicity.³⁾ Since an EVAL copolymer is capable of imbibing an adequate amount of water and shows good biocompatibility, it should be well adaptable as a matrix for implanted delivery systems for insulin.

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References and Notes

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