Controlled Release of Insulin from Injectable Biodegradable Triblock Copolymer Depot in ZDF Rats

Suna Choi[1] and Sung Wan Kim[1,2]

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Purpose. The purpose of this study was to design sustained release system which provides basal insulin release over a week by one injection in diabetic animals. For an effective injectable formulation and controlled release of insulin, a water soluble biodegradable triblock copolymer of PLGA-PEG-PLGA was used.

Methods. For *in vitro* release, samples were analyzed by reversed-phase high performance liquid chromatography. Animal studies using ZDF rats have been conducted to demonstrate the bioactivity of the released insulin. Insulin formulation was injected subcutaneously. At designated times, the blood glucose levels and insulin levels of the ZDF rats were measured.

Results. The *in vitro* release of zinc-complexed insulin showed no initial burst and demonstrated constant release rate with the duration of 14 days. Constant steady state plasma levels of exogenous insulin were detected for nearly two weeks indicating constant rate of insulin release *in vivo* upon single subcutaneous injection.

Conclusions. We conclude that it is feasible to achieve basal insulin levels over a week by a single injection of ReGel™ formulation. This will provide various advantages, including depot formation without surgery, easy sterilization, straightforward drug loading, simple dose adjustment, system biocompatibility with no inflammatory reaction, and no requirement of using organic solvents.

KEY WORDS: controlled drug delivery; biodegradable polymers; insulin delivery; diabetes.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease resulting from pancreatic beta cell dysfunction and insulin resistance. The disease and its complication is major leading cause of mortality in the United States (1). Its worldwide frequency is expected to grow rapidly, potentially reaching a total of 200–300 million patients in 2010 (2). Currently, approximately 40% of the 135 million people diagnosed with diabetes worldwide are receiving insulin as a component of their therapy (3). A patient with type 1 diabetes receives at least one subcutaneous insulin injection a day to lower glucose concentrations. Insulin treatment is also required to maintain glycemic control during later stages in type 2 diabetes. Generally, patients with diabetes mellitus need a relatively constant basal insulin supply to mimic a near-normal physiologic pattern of insulin secretion to improve their quality of life (4). The subcutaneous route, requiring single or multiple daily injections, is the main stay of conventional insulin therapy (5). It is inconvenient and painful, with poor patient compliance.

Sustained-release formulations of insulin have been reported for the last three decades. It would be advantageous to investigate formulations prepared with a biodegradable matrix. Goosen *et al.* (6) showed that insulin loaded into cross-linked albumin microbead implant was released for up to 3 weeks in diabetic rats (750 U/animal). However, this formulation was not injectable. Lin *et al.* (7) used hydroxyl propyl methylcelluclose phthalate (HP-55) and polylactic acid (PLA, MW 30,000) achieving normoglycemia for 5 days. Takenaga *et al.* (8) reported that injection of insulin-loaded microcapsules made of poly (D,L-lactic-co-glycolic acid) (PLGA, MW 6600) increased plasma insulin levels in streptozotocin-induced hyperglycemic rats (250 U/kg), but they did not report the glucose level of diabetic rats. Fabrication of this drug delivery system using such polymers involves use of organic solvents or heat that may result in protein denaturation and loss of bioactivity.

A water soluble, biodegradable triblock copolymer of poly((DL-lactide-co-glycolide)-b-ethylene glycol-b-(DL-lactide-co-glycolide)) was used in this study for an effective injectable formulation and controlled release of insulin. Since it possesses both thermosensitivity and biodegradability, it can act as an injectable implant system (9–11). In an aqueous solution, the copolymer, ReGel™ is a free flowing sol at room temperature and forms a gel with high viscosity at body temperature. The *in-situ* formed copolymer gel maintains its structural integrity up to 1 month (12).

A zinc salt has been used to reduce the solubility of zinc-insulin complex and to prolong the duration of release. It is well established that insulin at equilibrium conditions exists in monomeric, dimeric, and hexameric form. The last form prevails at higher Zn^{2+} concentration and has low solubility under certain conditions (13). In this study, we designed a sustained release system that provides basal insulin release over a period of 2 weeks by one injection in diabetic animals.

MATERIALS AND METHODS

Materials

Human insulin was purchased from Sigma (St. Louis, MO). Poly ((DL-lactic acid-co-glycolic acid)-b-ethylene glycol-b-(DL-lactic acid-co-glycolic acid)) triblock copolymer (PLGA-PEG-PLGA) (ReGel™) was supplied by MacroMed, Inc. (Salt Lake City, UT).

In vitro Release Study

The PLGA-PEG-PLGA triblock copolymer was dissolved in water at room temperature to make a 23 wt% solution. Insulin powder was loaded to a concentration of 6 mg/ml. For zinc-insulin, zinc carbonate was added (10 wt%) to the hydrogel solution. Then 1 ml of formulation was placed in a syntillation vial, incubated at 37°C for 2 min until gelled, and 10 ml of PBS release medium was added. Release medium samples were withdrawn and replaced periodically each day to maintain sink condition. Release samples were analyzed by reversed-phase high performance liquid chromatography (RP-HPLC) to measure the concentration of insulin. For RP-HPLC (SCL-10Avp, Shimadzu), C_4 column (Vydac) was used. The mobile phases were water and acetonitrile containing 0.1% TFA. Insulin was eluted by a gradient of 2% B per minute at the flow rate 1.2 ml/min.

[1] Center for Controlled Chemical Delivery (CCCD), Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, Salt Lake City, UT 84112-5820.

[2] To whom correspondence should be addressed. (e-mail: rburns@pharm.utah.edu)

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Animal Experiments

Twenty male Zucker Diabetic Fatty (ZDF) rats (410–460 g) were purchased from Charles River Laboratories (Wilmington, MA) at 12 weeks of age. All animals used in this study were housed according to the principles established for care and use of laboratory animals (21–23°C, 12-12 h light-dark cycle). They were fed *ad libitum* with Purina 5008 (6.5% fat). The experimental protocols concerning the use of laboratory animals were reviewed and approved by the Institutional Animal Care and Use Committee of University of Utah.

The experimental animals were divided into two groups. The ZDF rats were fasted overnight and anesthesia was induced by intramuscular injections of Pentobarbital (60 mg/kg). The PLGA-PEG-PLGA triblock copolymer was dissolved in water at room temperature to make a 23 wt% solution and this solution was sterile filtered. For the first group, 1 ml of polymer solution was injected. For the second group, insulin powder was added to a concentration of 6 mg/ml, followed by addition of powdered zinc carbonate (10 wt%) to the hydrogel solution using a clean biosafety cabinet. One hour after the anesthesia, 1 ml insulin formulation was injected subcutaneously (n = 5). At designated times (1, 3, 6, and 12 h, every day for 2 weeks), 600 μl of blood was obtained from the tail vein of ZDF rats. Using Accucheck-Instant (Boehringer Mannheim, Indianapolis, IN, USA), the blood glucose levels of the ZDF rats were measured. The plasma insulin levels were determined using commercial insulin radioimmunoassay kit (ICN Pharmaceuticals, Costa Mesa, CA, USA).

RESULTS AND DISCUSSION

The triblock copolymer used in the study is a hydrophilic/hydrophobic balanced polymer. This polymer was designed based on the fact that hydrophobic interactions are enhanced at elevated temperatures and the hydrophobic domain of the copolymer forms physical crosslinks (or aggregate) (10) which enables the gel state at 32°C for the particular polymer used in this study.

Figure 1 displays the results of insulin release from hydrogel depot *in vitro*. It should be noted that there is no initial burst of insulin from zinc-complexed insulin/ReGel™ formulation. The cumulative amount of insulin released over time is shown in Fig. 1. Zinc plays an important role in the sustained release of insulin. Zinc complexation provided a means to stabilize this physically, chemically unstable polypeptide, as well as reduction of initial burst. The release of zinc-complexed insulin/ReGel™ formulation shows more or less constant release kinetics with the duration of 14 days. Without zinc, the release profile exhibited higher burst effect and shorter duration.

An animal study using Zucker Diabetic Fatty rats was performed with insulin (10 wt% zinc carbonate)/ReGel™ formulation. A RIA analysis of plasma samples for insulin content was performed at the pre-selected time points. Figure 2 and Fig. 3 show plasma insulin and blood glucose levels. The insulin level was almost constant (4–10 μg/L) till day 10 and dropped at day 12. Thus, insulin release *in vivo* from ReGel™ depot was constant until day 10 following subcutaneous injection. These findings *in vivo* correlate with *in vitro* studies, which showed constant release for 2 weeks. The plasma insulin levels correlated with the blood glucose levels. The blood glucose level in diabetic animals dropped to euglycemic range (80–125 mg/dL) during insulin release period. This result directly indicates that the insulin released from the thermosensitive biodegradable hydrogel (ReGel™) formulation is bioactive, and can control blood glucose levels in type 2 diabetic animals. The safety profile of ReGel™ was investigated and there was no inflammatory reaction at the injection site after injection.

Patients with diabetes mellitus need a constant basal insulin supply to mimic a near-normal physiologic pattern of insulin secretion to improve glycemic control (4). The targets for blood glucose control, as recommended by the American Diabetes Association, are fasting preprandial blood glucose levels between 80 mg/dL and 120 mg/dL, bedtime glucose levels between 100 mg/dL and 140 mg/dL. Attempts to mimic basal insulin secretion have been difficult because insulin shows initial burst after injection and short durations of action. The insulin levels achieved with ReGel™ system were able to control hyperglycemia of diabetic subjects. By using this system, type 1 diabetic patients can be treated with less dose of short acting insulin. For type 2 diabetic patients, it can be more effective when they need combination therapy with oral agents. Overall, a controlled release of insulin can improve patient compliance and therapy.

We conclude that it is feasible to achieve base line insulin

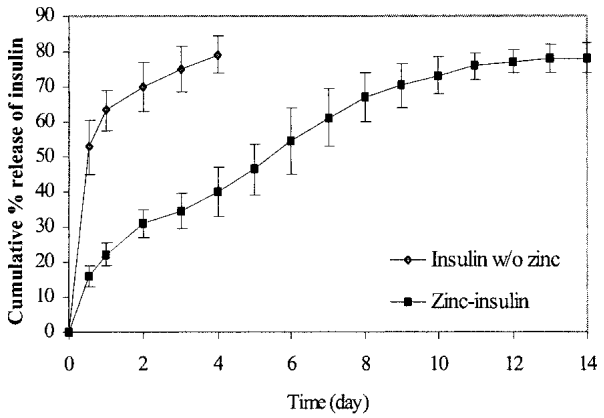

Fig. 1. Cumulative % amount of released insulin from ReGel™ formulation *in vitro* at 37°C. The graph represents the average ± SE, and each group was composed of 3 sets.

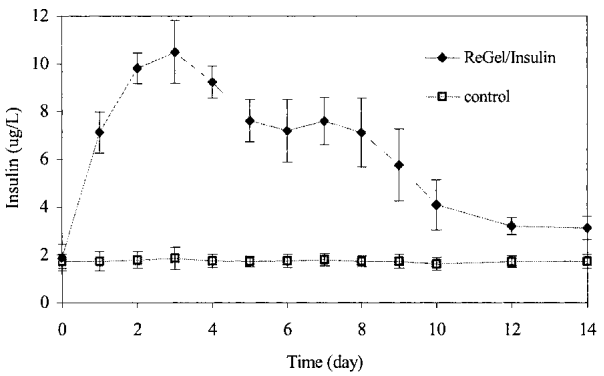

Fig. 2. Plasma insulin levels in ZDF rats. The graph represents the average ± SE, and each group was composed of five rats.

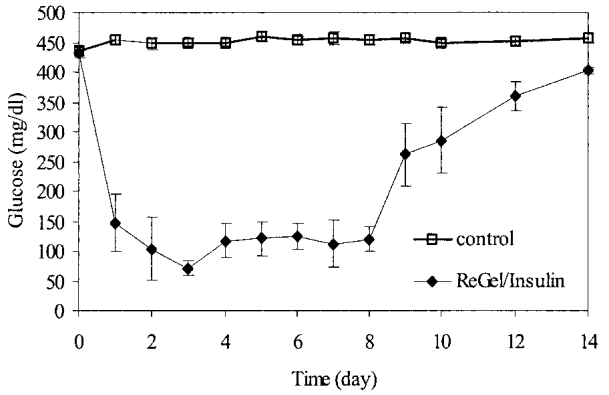

Fig. 3. Blood glucose levels in ZDF rats. The graph represents the average ± SE, and each group was composed of five rats.

levels *in vivo* over 1 week by a single injection of ReGel™ formulation. There are various advantages of the system, including depot formation without any surgical procedure, easy sterilization by filtration, straightforward drug loading to the polymer solution, simple dose adjustment, system biocompatiblility with no inflammatory reaction due to biodegradablility, as well as no requirement of using organic solvents during fabrication.

CONCLUSIONS

The thermosensitive, biodegradable triblock copolymer (ReGel™) was used as an injectable depot for sustained release of insulin. The *in vitro* release of zinc-complexed insulin from ReGel™ showed no initial burst and demonstrated constant release rate. Animal studies using Zucker Diabetic Fatty rats have been conducted to demonstrate the bioactivity of the released insulin. Constant steady state plasma levels of exogenous insulin were detected for almost 2 weeks, indicating constant rate of insulin release *in vivo* upon single subcutaneous injection. Sustained insulin release from ReGel™ depot as the delivery vehicle kept blood glucose levels in euglycemic range in type 2 diabetic animals for a 2-week period.

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