Controlled Release of Insulin from Injectable Biodegradable Triblock Copolymer

Young Jin Kim,[1] Suna Choi,[1] Jae Joon Koh,[1] Minhyung Lee,[1] Kyung Soo Ko,[1] and Sung Wan Kim[1,2]

Received December 14, 2000; accepted January 4, 2001

KEY WORDS: biodegradable; thermosensitive; hydrogel; insulin; drug-delivery.

INTRODUCTION

Protein drug delivery has become an important area of research due to the large number of recombinant proteins that are now being investigated for therapeutic applications. However, proteins have very short *in vivo* half-lives and they require multiple injections to achieve the desired therapeutic effect. One of the way to increase the therapeutic efficiency of these polypeptides is encapsulating them in a sustained dosage form that is capable of releasing the macromolecule continuously and at a controlled rate (1).

Typically, proteins have been loaded into the polymers like poly(lactic acid) (PLA), poly(lactic acid-co-glycolic acid) (PLGA) and PLGA-PEG-PLGA (2,3). Fabrication of drug delivery systems using such polymers involves the use of organic solvents or heat that results in protein denaturation and loss of bioactivity. Many problems in loading proteins can be overcome by the use of polymeric hydrogels such as the Poloxamer series which have sol to gel transition characteristics and are soluble in water (4). However, they are not considered an optimal system for the delivery of protein drugs because Poloxamers are toxic and are not biodegradable.

For an effective injectable formulation and controlled release of insulin, a water soluble, biodegradable triblock copolymer of poly((D,L-lactide-co-glycolide)-*b*-ethylene glycol-*b*-(D,L-lactide-co-glycolide) was used in this study as a new injectable implant system that possesses both thermosensitivity and biodegradability (5,6). The copolymer is a free flowing sol below 15°C in aqueous solutions and forms a high viscosity gel at body temperature. The known gelling polymer, Poloxamer formed the gel, which is water soluble and dissolved in a few days at most. However, the ReGel® hydrogel system is a water insoluble gel that can maintain its integrity for more than one month (7). Therefore, it is applicable for an injectable long-term drug delivery system(8,9).

Drug release from the hydrogel is affected by several factors such as pore size, degradability, size, hydrophobicity, concentration of a drug, and the presence of specific hydrogel-drug interactions. Initially, the release mechanism from a biodegradable hydrogel is limited by the drug's diffusivity. Then, a combination of diffusion and degradation processes controls the drug's release from the polymeric matrix (10). In this study, human insulin was used as a target drug. Diabetes mellitus is a serious pathological condition responsible for major health care problems all around the world costing billions of dollars annually. In the United States, it represents the fourth leading cause of death.

Diabetes mellitus also leads to severe complications such as kidney disease, retinopathy, neuropathy, leg or foot amputations and heart disease (11). As a consequence of poor oral bioavailability and current lack of alternative delivery routes, insulin is presently administered parenterally. The subcutaneous route, requiring single or multiple daily injections, is the mainstay of conventional insulin therapy (12). In this study, we designed the sustained release system, which provides basal line insulin release for duration of over several weeks by one injection. Human insulin was entrapped in the hydrogel in order to sustain its release in a subcutaneous insulin delivery system. We tried to modify the association states of insulin by zinc in order to inhibit the initial burst effect and obtain constant release rate. At otherwise equivalent conditions, insulin associates from monomer and dimer to hexamer with increasing zinc concentration (13). Insulin samples with different zinc contents exhibit different release profile due to association-state differences within the hydrogel.

MATERIALS AND METHODS

Materials

Poly((D,L-lactic acid-co-glycolic acid)-*b*-ethylene glycol-*b*-(D,L-lactic acid-co-glycolic acid)) (1500-1000-1500) triblock copolymer (ReGel®) was supplied by MacroMed, Inc. (Salt Lake City). Recombinant human insulin was purchased from Sigma (St.Louis, MO).

Sol-Gel Transition

Triblock copolymer solutions were prepared by dissolving the polymers in cold water at 4°C to make 15, 23 wt% solutions. The sol-gel transition temperature was measured by increasing the temperature at 2°C increments. A UV cuvet was immersed in a water/glycerol bath at each temperature for 5 min. The sol-gel transition was monitored by its absorbance at 500 nm using a UV spectrometer (Lambda 19, Perkin Elmer).

In Vitro Release Test

The PLGA-PEG-PLGA triblock copolymer was dissolved in the water at 5°C to make a 23 wt% solution. Insulin solutions were prepared in buffer (isotonic 10 mM PBS, pH 7.4) to a concentration of 5.04 mg/ml and zinc was added (0.0, 0.2 wt%) to the hydrogel solution. Then 2 ml of each formulation were placed in vials (n = 5), incubated at 37°C until gels formed (2 minutes), and 10 ml of PBS solution was added as a release medium. The release medium was shaked in the water bath at 30 strokes per minute to ensure adequate mixing. Samples from the release medium were withdrawn and replaced immediately with fresh buffer to keep the sink condition. They were analyzed by reversed-phase high perfor-

[1] Center for Controlled Chemical Delivery, University of Utah, 30S 2000E RM201, Salt Lake City, Utah 84112-5820.

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

0724-8741/01/0400-0548$19.50/0

mance liquid chromatography (RP-HPLC) to measure the concentration of insulin. RP-HPLC (SCL-10Avp, Shimadzu) was equipped with a C_4 column (5 μm particle, 250 × 4.6 mm i.d.; Vydac), which was previously equilibrated and the detection wave length was 220 nm. The mobile phases were water and acetonitrile with a gradient flow and a flow rate of 1.2 ml/min. The concentration of acetonitrile was linearly increased from 25% to 80% for 20 minutes. The detection limit was about 1 μg/ml.

In Vivo Release Test

Male Sprague-Dawley rats (350 gram) were kept under specific pathogen free conditions in the animal facility. The SD rats were fasted overnight and anesthesia was induced by intramuscular injections of Pentobarbital (6 mg/kg). The triblock copolymer aqueous solution was prepared by the same method as the *in vitro* test. Human insulin was added into the polymer solution with zinc (10 IU/ml). Thirty minutes after the anesthesia, 0.6 ml insulin solution were injected subcutaneously (n = 5). At designated times, 300 μl of blood were obtained from the tail vein of SD rats after overnight fasting. The plasma insulin levels were determined by using insulin radioimmunoassays kit (ICN Pharmaceuticals, Costa Mesa, CA). The concentration of insulin in the hydrogel matrix is different for *in vitro* and *in vivo* studies. However, we kept the ratio of insulin and zinc in both release studies. We loaded enough insulin to investigate through HPLC (detection limit: 1 μg/ml) for *in vitro* study. For *in vivo* study, we designed the amount of insulin, which is the necessary amount for the rat model for 15 days.

RESULTS AND DISCUSSION

The properties and synthesis of the ReGel® A-B-A triblock copolymer used in these studies has been reported previously (7), however the key features are repeated for clarity. The ReGel® triblock copolymer had a weight average molecular weight of 4200 Da as measured by GPC using polyethylene glycol standards. The A-blocks were synthesized from the ring-opening polymerization of D,L-lactide and glycolide in the mole ratio of 75:25, respectively, in the presence of polyethylene glycol (PEG 1000). The molecular weights of the A-blocks were each approximately 1600 Da, with the molecular weight of the B-block at 1000 Da. The A-blocks were covalently coupled to the B-block via ester linkages. The degradation profile of this copolymer at 37°C has been reported previously (8,9), with complete degradation to lactic acid, glycolic acid, and PEG 1000 occurring in 4 to 6 weeks. ReGel® has a hydrophilic-hydrophobic group inside the copolymer. The hydrophilic-hydrophobic copolymer formed the micelle as the hydrophobic interaction was enhanced with the increasing temperature. The gel state was above the critical micelle concentration. And a higher concentration of copolymer makes more micelles than a lower concentration of copolymer at the same temperature. The sol-gel transition occurred at a lower temperature with a higher concentration of copolymer. The aqueous solution of ReGel® was investigated by UV spectrometer to determine the sol-gel transition temperature. Figure 1 shows a sol-gel transition temperature of 15–20°C with the concentration variation of ReGel® solution. The ReGel® solution is a free flowing sol below 15°C and

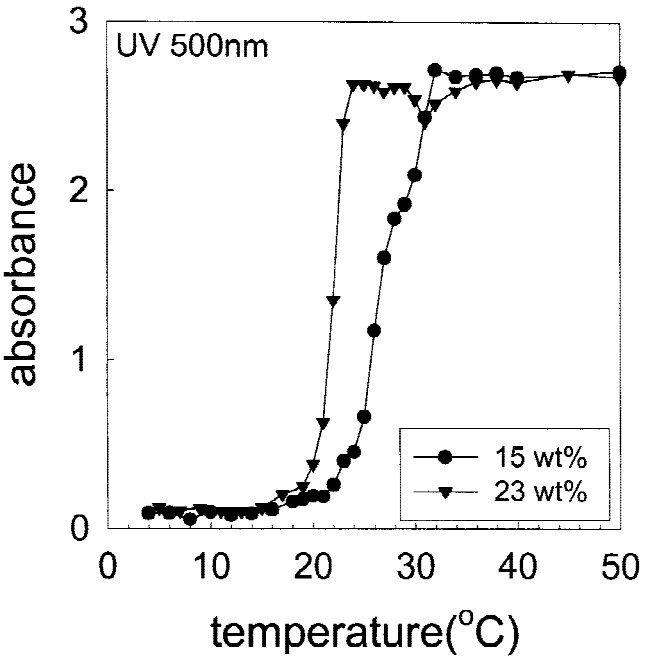

Fig. 1. Sol-gel transition curve of ReGel® aqueous solution by UV spectrometer.

forms a high viscosity gel at body temperature in aqueous solution. At low temperatures (<15°C), the solution can be formulated with a labile drug such as a bioactive protein and the formulated solution can be injected to the body for the controlled release of macromolecular drugs.

Figure 2 shows the result of insulin release from the hydrogel *in vitro*. There was no initial burst effect of the insulin release from the ReGel® formulations. The ReGel® hydrogel system is thought to have a core-shell structure in an aqueous environment (8). The hydrophilic PEG occupies the shell region and hydrophobic PLGA hides into the core in order to

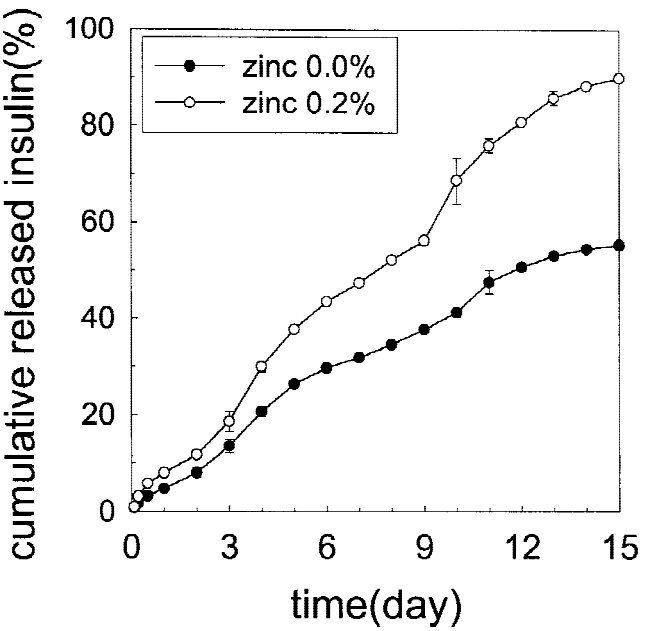

Fig. 2. Cumulative amount of released insulin from ReGel® formulation *in vitro* test (n = 5).

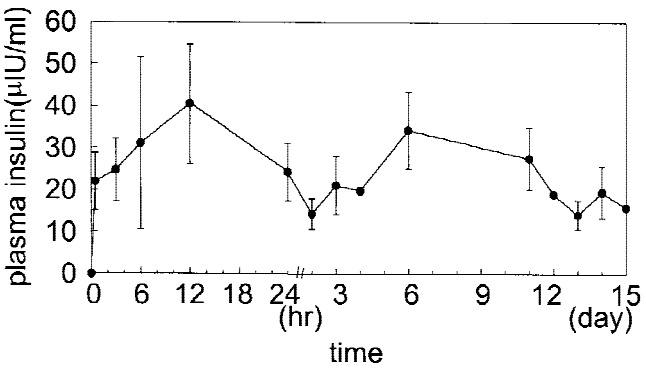

Fig. 3. Plasma insulin level of SD rat *in vivo* test (n = 5).

decrease the free energy. Assuming a domain structure of the hydrogel, the partitioning of drug between the hydrophilic domain and the hydrophobic domain was considered. Insulin is a hydrophobic drug and may mostly be located inside the hydrogel network. Drug release from the hydrophilic domain can be described by diffusion. It represents the release profile till day 7. After day 7 the hydrogel network, especially hydrophobic PLGA started to degrade so that the diffusion and degradation governed the release profile of day 7 to day 15. Generally, insulin in the solution has the various association states such as monomer, dimer, hexamer, and aggregate (13). The insulin is readily aggregated in the hydrogel solution, which is slightly acidic. The zinc ion can help insulin to form the stable hexameric state. It is thought that insulin without zinc formed an aggregation state inside the gel. The aggregated insulin may not diffuse fast from the ReGel® formulation, which presented the slower release (60% after 15 days). Insulin with 0.2 wt% zinc formed the hexameric state. The release profile of the insulin with zinc showed a constant (zero-order) release rate and almost 90% of the initial amount was released over 15 days. An animal study using Sprague-Dawley rats was performed with insulin (0.2 wt% zinc)/ReGel® formulation in order to verify the *in vitro* result. RIA analysis of insulin was performed on plasma samples at the designated time. Figure 3 shows the insulin in the plasma sample of the designated time. There have been steady amounts of insulin secretion from ReGel® formulation up to day 15 after a subcutaneous injection. In contrast, plasma insulin levels of the control group (ReGel®) showed undetectable concentrations. Current protocol of insulin supplementation relies on daily or continuous subcutaneous injection of insulin to meet the basal and postprandial insulin requirements. In this study, ReGel® formulation maintains insulin secretion up to 15 days, which can enable diabetic patients to reduce the number of insulin injection twice a month for basal insulin requirements.

CONCLUSIONS

An ABA triblock copolymer composed of PLGA and PEG (ReGel®) was used as a drug delivery carrier for the continuous release of human insulin. The ReGel in an aqueous solution is a free-flowing sol at room temperature and becomes a gel at body temperature. The release of human insulin from ReGel® showed no initial burst and a constant release (zero–order) rate *in vitro* test. It was necessary to modify the insulin's zinc content to 0.2 wt% in order to get a maximum release rate. Animal studies using SD rats have been investigated to verify, *in vivo,* the release profile of insulin from the ABA block copolymer. There have been steady amounts of insulin secretion from the ReGel formulations up to day 15 of the subcutaneous injections. It is feasible to release basal requirement of insulin for 15 days with one injection.

ACKNOWLEDGMENTS

The authors thank to Dr. Gaylen Zentner at MacroMed, Inc. for the supply of ReGel®.

REFERENCES

1. S. Cohen, T. Yoshioka, M. Lucarelli, L. H. Hwang, and R. Langer. Controlled delivery systems for proteins based on poly-(lactic/glycolic acid) microspheres. *Pharm. Res.* **8**:713–720 (1991).
2. E. A. Pec, Z. G. Wout, and T. P. Johnston. Biological Activity of urease formulated in poloxamer 407 after intraperitoneal injection in the rat. *J. Pharm. Sci.* **81**:626–630 (1992).
3. L. Youxin and T. Kissel. Synthesis and properties of biodegradable ABA triblock copolymers consisting of poly(L-lactic acid) or poly(L-lactic-co-glycolic acid) A-blocks attached to central poly-(oxyethylene) B-blocks. *J. Control. Release* **27**:247–257 (1993).
4. M. Malstom and B. Lindman. Self-assembly in aqueous block copolymer solutions. *Macromolecules* **25**:5440–5445 (1992).
5. B. Jeong, Y. H. Bae, D. S. Lee, and S. W. Kim. Biodegradable block copolymers as injectable drug-delivery systems. *Nature* **388**: 860–862 (1997).
6. B. Jeong, D. S. Lee, J. Shon, Y. H. Bae, and S. W. Kim. Thermoreversible gelation of poly(ethylene oxide) biodegradable polyester block copolymers. *J. Polym. Sci.: Part A: Polym. Chem.* **37**:751–780 (1999).
7. R. Rathi and G. Zentner. Biodegradable, low molecular weight triblock poly(lactide-co-glycolide) polyethylene glycol copolymers having reverse thermal gelation properties. *U.S. Patent* 6,004,573.
8. B. Jeong, Y. H. Bae, and S. W. Kim. Drug release from biodegradable injectable thermosensitive hydrogel of PEG-PLGA-PEG triblock copolymers. *J. Control. Release* **63**:155–163 (2000).
9. B. Jeong, Y. H. Bae, and S. W. Kim. *In situ* gelation of PEG-PLGA-PEG triblock copolymer aqueous solutions and degradation thereof. *J. Biomed. Mater. Res.* **50**:171–177 (2000).
10. R. Baker. In *Controlled Release of Bioactive Materials,* Academic Press, New York, 1980.
11. M. Baudys and S. W. Kim. Preface. *Adv. Drug Delivery Rev.* **35**:141–142 (1999).
12. M. Hinchcliffe and L. Illum. Intranasal insulin delivery and therapy. *Adv. Drug Deliv. Rev.* **35**:199–234 (1999).
13. J. Brange, and L. Langkær. In Y. J. Wang, and R. Pearlman (eds.), *Stability and Characterization of Protein and Peptide Drugs: Case Histories*, Plenum Press, New York, 1993 pp. 315–350.