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SHORT CONTRIBUTION

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Triblock copolymer Pluronic®F127 sustains insulin release and reduces initial burst of microspheres—in vitro and in vivo study

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Abstract In many cases, initial release of drugs from microparticles is undesirable. In the present study, Pluronic®F127, which shows thermosensitive characteristic, was used for controlling both the initial release and the sustained release of insulin from microspheres. Calcium-alginate insulin microspheres were prepared by emulsion technology and dispersed in a thermosensitive semisolid gel, the PF127. In vitro study demonstrated that PF127 could protect the activity of insulin in some extent and control the initial release and sustained release of insulin well. The results of in vivo hypoglycemic experiment carried out on diabetic rats also displayed a well correlation with in vitro release patterns. The results suggested that PF127 could sustain insulin release through a subcutaneous injection, and this material was available to deliver insulin aiming for prolonged and smooth hypoglycemic function.

Keywords Pluronic®F127 · Alginate calcium · Insulin · Initial release · Microsphere · Sustained release

Introduction

It is well known that biomedical materials attracted more and more attention in drug delivery fields [1–3]. Pluronic®, which is also called poloxamer, is a class of nonionic, surface-active triblock copolymers with different molecular weights. The structure of Pluronic® is polyethylene oxide (PEO)–polypropylene oxide (PPO)–PEO block copolymer or polypropylene oxide (PPO)–polyethylene oxide (PEO)–PPO blocked. Pluronic®127, composed approximately of 70% ethylene and 30% propylene oxide with a molecular weight of 115,000 Da, is a kind of temperature-sensitive polymer and demonstrates a desirable reversible gel character with change of temperature at concentrations of 20% or higher. At room temperature (25 °C), PF127 exists as a kind of viscous liquid, which can transform into a semisolid gel at body temperature (37 °C). Thus, it is possible to design a formulation with PF127, which is liquid at lower temperatures but can provide a depot of drug at the injection site once injected. PF127 has been used in controlling drug release [4, 5], cell culture [6], and other fields. Zhang et al. [7] investigated the release character of ceftiofur in PF127 gel. Shishido [8] used the gel to control a prodrug of nitric oxide. Kim et al. also complexed rhEGF into HP-β-CD and controlled by using PF127 [9]. All of the above works demonstrated the sustained drug-release effect of PF127.

Alginate gel materials, which have always been used for preparing microparticles, are hoped for sustaining drug delivery [10]. However, initial burst is a big problem for

microparticles [11]. In other words, burst dose of drug may lead to an undesirable effect considering the toxicity and therapeutic window.

In this study, insulin was employed to detect the effect of PF127 on the burst release of drug from the microparticles. Insulin, which composed of two peptide chains, is a kind of preferred drug for diabetes. However, this protein is unstable when used in vivo due to the enzyme degradation. Up to now, large amount of work has been devoted into the preparation of insulin delivery system for prolonging its activity in vivo and maintaining the hypoglycemic effect [12–14]. Though many kinds of microparticles have been prepared and some ones showed protection effect on insulin, how to control the initial burst is still unfathomed.

In our experiments, the thermosensitive characteristic of PF127 was utilized, and both insulin–PF127 and insulin calcium-alginate microshpheres–PF127 were prepared. Subsequently, the effect of PF127 on the initial release and sustained release of insulin were evaluated. Furthermore, in vivo hypoglycemic effects of these preparations were also investigated.

Experimental

Materials

The following products were used: insulin (Jiangsu Wanbang Biochemistry, CHN), Pluronic®F127 (PF127, BSAF GER), alloxan (Sigma, USA), alginate (Shanghai Chemical Reagent, China National Pharmaceutical Group, CHN), glucose kit (Shanghai Institute of Bioproduction, CHN); other reagents were of analytical purity. Scanning environmental microscope (XL30, PHILIPS), homogenizer (FJ-200 Shanghai Species and Model Factory, CHN), Agilent 1100 series HPLC instrument (Agilent Technologies, USA), and Malvern Zetasizer 3000HS (Malvern UK) were also used.

Male Sprague–Dawley rats (6 weeks), weighing 240± 40 g, were supplied by the animal center of Zhejiang Academy of Medical Sciences. All the procedures were in strict compliance with the People's Republic of China legislation on the use and care of laboratory animals and with the guidelines established by the Institute for Experimental Animals of Zhejiang University and were approved by the university committee for animal experiments.

Preparation of PF127 solution

A quantity of PF127 powder was weighed, mixed with distilled water, and stored at 4 °C until it was dissolved. The final concentration was 30% (*w*/*v*).

Preparation of insulin calcium-alginate microspheres and morphology observation

The preparation was modified from the method previously reported [10, 15]. In brief, 70 mg of insulin was precisely weighed. After adding 2 ml of 0.01 mol/l HCl, 8 ml of Tris–HCl buffer (pH 7.4) was added to equilibrate the pH level. Then, 10 g of 2% sodium alginate was added into the insulin suspension and homogenized (8,000 rpm×3 min) after adding 40 ml of octane and 5% Span80. The emulsion was formed after adding 3 ml of 30% Tween80, 8 ml of 8% $CaCl_2$, and 40 ml of isopropane under homogenization at 8,000 rpm. Then, the emulsion was separated by centrifugation. The calcium-alginate microspheres were collected and lyophilized.

The morphology of microspheres was observed by scanning electron microscope (SEM), and granulate diameter was detected by a Zetasizer.

Detection of insulin by HPLC

Agilent 1100 series LC solvent delivery system and ultraviolet detector were used in this study. The working conditions were listed as follows: Hypersil ODS column (150 mm×4.6 mm×5 μm) (Elite, Dalian, China), mixture of 0.025 mol l^{-1} sodium sulfate buffer (pH 2.0) and acetonitrile (72:28) as mobile phase, flow rate: 1 ml min^{-1}, column temperature 40 °C, and detection wavelength: 214 nm [16].

Detection of entrapment efficiency (EE)

A quantity weight of microspheres was dispersed in PBS (pH 7.4) and centrifuged (3,000 rpm×5 min). The supernatants were collected and added with fresh media. After 4 h, extraction media were merged and insulin was detected after filtration with a 4.5-μm polycarbonic ester membrane.

$$\mathrm{EE} = \text{actual loading drug}/\text{theoretic drug quantity} \times 100\%$$

Investigation on insulin stability in PBS and PF127 gel

Stability in PBS

Five hundred micrograms per milliliter of insulin was prepared and placed in PBS at 37 °C; a quantity of sample was withdrawn at intervals and detected by HPLC.

Stability in PF127

Five hundred micrograms per milliliter of insulin was dissolved in 30% PF127 and gelled at 37 °C. At certain

intervals, the gel was cooled to become a solution, and a quantity of sample was taken out and detected.

Insulin release in vitro

Insulin release from calcium-alginate microspheres

A quantity of insulin microspheres was weighed into a release tube; 3 ml of PBS was added as a release media. Release tube was put in a swing bed of 37 °C with a shaking rate of 100 rpm. At predetermined time point, 1 ml of release media was withdrawn and replenished simultaneously. Insulin was detected by HPLC.

Insulin release from PF127 gel

Membraneless method was used in this study [17]. Shortly, insulin solution was dispersed in PF127, and the final concentration of PF127 was 30%. Three milliliters of PF127 solution was transferred into a tube which was fixed on a 37 °C swing bed and then gelled. One milliliter of PBS (pH 7.4) was added to the surface of the gel, then the tube was shaken at a rate of 100 rpm. At certain intervals, the supernatant was withdrawn, and 1 ml of PBS was replenished.

Insulin microspheres release from PF127

A quantity of insulin microspheres was weighed and suspended with 0.5 ml of distilled water. Two milliliters of PF127 solution was mixed with the suspension steadily, and the final concentration of PF127 was 30%. The following steps are the same to the method mentioned above.

In vivo experiment on hypoglycemic effect of insulin preparation

Hyperglycemia model of rats

Male Sprague–Dawley rats were intraperitoneally injected with alloxan (140 mg kg^{-1}) twice in 24 h. After 7 days, the blood sugar concentrations were determined. Those which blood sugar higher than 11 $mmol \cdot l^{-1}$ were used as the models [18]. Animals were housed in air-conditioned room in accordance with the guidelines for care and use of laboratory animals.

Hypoglycemic experiment

The hyperglycemic rats were divided into three groups randomly and were subcutaneously injected with insulin solution (2 IU/kg), insulin–PF127 solution (4 IU/kg), and insulin microspheres–PF127 solution (4 IU/kg), respectively [19]. At certain time points, the blood samples were obtained from the tails. The plasma was separated from a heparinized tube, and the blood sugar concentration was determined by a glucose oxidase kit according to the manufacturer's instruction.

Results

Morphology investigation and entrapment efficiency of insulin microspheres

The mean diameter of the alginate calcium was 1.8 μm measured by Zetasizer. SEM photo (Fig. 1) indicates that the microspheres are sphere-shaped and have miniporous surface.

The EE of insulin microsphere detected by HPLC was 48.64±5.39%.

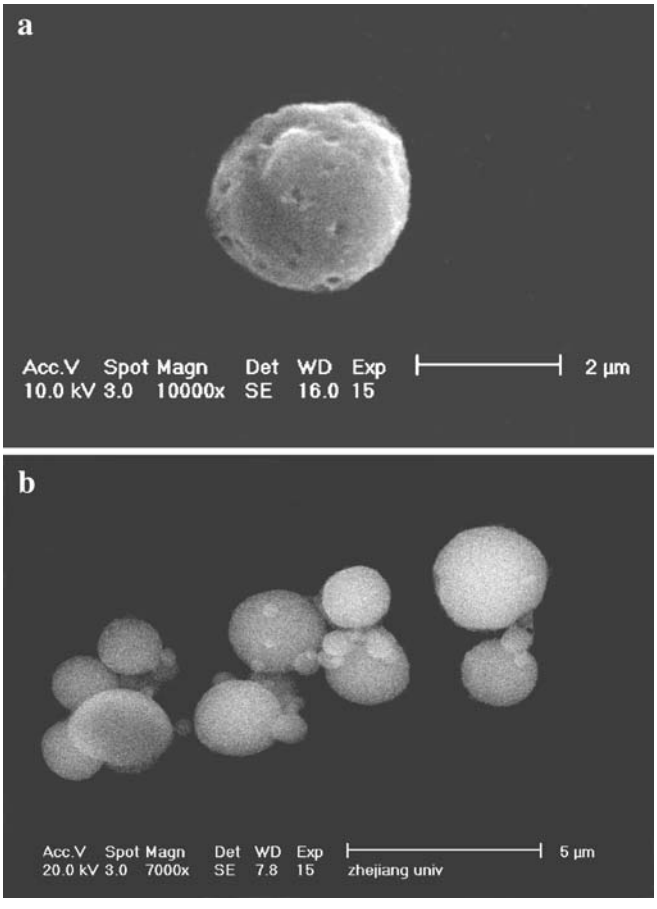

Fig. 1 The SEM photo of insulin calcium-alginate microspheres

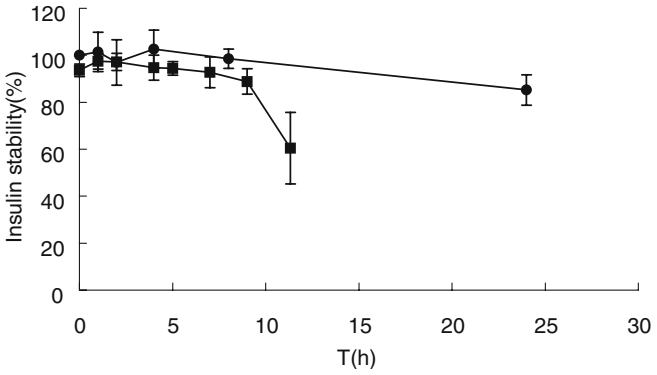

Fig. 2 The stability of insulin in PBS (pH 7.4) solution and PF127 gel (mean±SD, *n*=3). PF127 gel (-•-), PBS (pH 7.4) solution (-▪-)

Insulin stability in different media

It can be concluded from the results listed in Fig. 2 that PF127 could improve the stability of insulin. The concentration of insulin was reduced to 60.74±15.26% compared to the initial level after 12 h exposed in PBS (pH 7.4). In contrast, insulin encapsulated in PF127 maintained the concentration to 85.23±6.49% after 24 h.

In vitro release of insulin preparations

The results of in vitro release experiments (Fig. 3) demonstrated that PF127 notably inhibited the burst release of insulin from microspheres. While there is an initial burst of insulin attached to 46.24±17.81% at the first sampling point and 95.68±17.72% at 7 h in calcium-alginate microspheres, the burst release from microspheres encapsulated in PF127 gel was reduced, and the cumulative release of insulin after 24 h was only 16.17±1.85%. On the other hand, release character of insulin in PF127 gel was similar to that of microspheres in PF127 gel, suggesting that a sustained release character was mainly controlled by the gel.

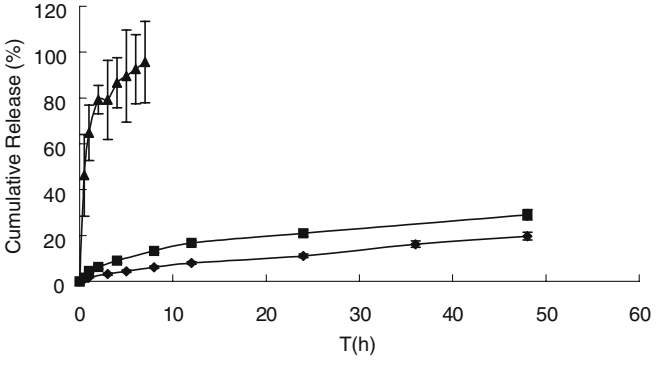

Fig. 3 In vitro release of insulin from different preparations (mean±SD, *n*=3). Insulin alginate-calcium microspheres (-▲-), insulin–PF127 (-▪-), insulin microspheres–PF127 (-♦-)

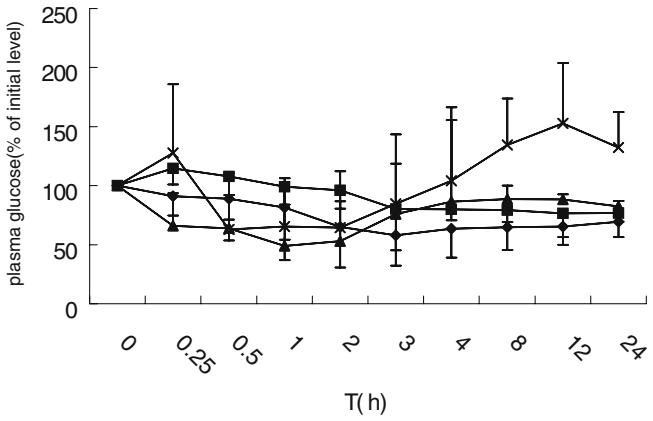

Fig. 4 Hypoglycemic effects of insulin and its preparations on diabetic rats (mean±SD, insulin solution group *n*=4,). insulin solution (-×-), insulin–PF127 (-•-), insulin microspheres (-▲-), insulin microspheres–PF127 (-▪-)

Hypoglycemic experiment in vivo

Both insulin solution and its alginate microspheres resulted in a sharp reduction of plasma glucose at the beginning phase after administration. The solution only maintained the hypoglycemic effect for 2.5 h, and the glucose level also increased after 2 h with the treatment of microspheres. For PF127-controlled preparations, a smooth and retarded hypoglycemic effect appeared in diabetic rats. In PF127-controlled insulin group and PF127-controlled microspheres group, the lowest blood sugar appeared at 3 h (57.88±25.79%) and 12 h (76.60±20.17%). Both the preparations at least maintained the hypoglycemic effect to 24 h. The results were coincidence with the in vitro results and shown in Fig. 4 and Table 1.

Discussions

Insulin always loses its activity by self-aggregation [20]. However, from the results shown in Fig. 2, PF127 improved the stability of insulin. As a kind of block copolymer of PEO–PPO, PF127 owns a considerable hydrophilic property. This semisolid gel might be used as a

Table 1 Comparison of hypoglycemic effect of insulin and its preparations (mean±SD, *n*=4)

	Hypoglycemic effect appearance time (h)	Maintenance of hypoglycemic effect (h)
Insulin solution	0.5	2.5
Insulin alginate-calcium microsphere	0.25	24
Insulin in gel	0.25	24
Insulin microsphere in gel	3	24

brace to support insulin molecules, and to inhibit the molecules to contact with each other, subsequently reducing the formation of aggregates.

On the other hand, how to control the release, especially the undesirable initial release, of insulin from preparations to reduce the risk of overhypoglycemic effect in vivo is a big challenge. In vitro release data showed the satisfactory sustained release character to both insulin and its microspheres controlled by PF127 gel (Fig. 3). Furthermore, the traditional and puzzling burst release of protein from microspheres disappeared in this delivery system. The release curve showed that nearly half of the loading drugs were released from microspheres without the protection of gel within 0.5 h, indicating that some amounts of insulin were adsorbed at the porous surface of microspheres, which had also been demonstrated by the SEM photo (Fig. 1). However, when dispersed in the gel which acted as a barrier to prevent the burst release of protein from surface, the critical step of drug release was gel corrosion process, and the slow and stable corrosion of gel induced the sustained release of drug.

We also observed that insulin calcium-alginate microspheres demonstrated a comparative slower release than that of insulin when both were dispersed in PF127 gel. The results suggested that both PF127 and alginate gel play roles in controlling the release of protein. Different from PF127-controlled insulin, drugs in PF127-controlled microspheres may release in two phases; this leads to a slower release result.

Subsequently, the hypoglycemic experiment in vivo was carried out, and a well correlation between in vitro and in vivo was observed in the preparations controlled by PF127. The results demonstrated that the rats treated with insulin solution presented a reduction of sugar in plasma 0.5 h after administration, and the level of sugar began to increase after 3 h. In contrast, compared to insulin microspheres and insulin solution, PF127-controlled preparations prolonged the hypoglycemic effect obviously. Moreover, these preparations showed a retarded pharmacodynamics emergence time in contrast to insulin solution and microspheres (Table 1). Insulin alginate microspheres showed a remarkable hypoglycemic effect shortly after the administration, indicating the initial burst of surface-adsorbed drug. However, after 2 h, the blood sugar was increased. In contrast, adding of PF127 controlled the insulin and its microspheres to present a smooth hypoglycemic curve: did not decrease blood sugar sharply at the first release stage and did maintain hypoglycemic effect for a relative long term. The results are consistent with in vitro released data.

As a thermosensitive intelligent polymer, poloxamer 407 has been demonstrated that it could control both the initial release and the sustained release of protein from microparticular preparations. Furthermore, its protecting ability for unstable large weight molecules is also favorable. Though approaches to improve the intensity and chemical property of PF127 are expected, poloxamer is a promising candidate to be widely used in developing novel drug delivery system.

Conclusion

Controlled by a thermosensitive hydrogel, the PF127, insulin and its calcium-alginate microspheres showed a sustained release effect, and initial burst of microspheres was reduced markedly. The effect was also confirmed by the in vivo hypoglycemic study on diabetic rats. The approach carried out in the present study may be also suitable for other microparticular preparations to obtain a prolonged release without burst. It is also noticeable that PF127 could improve the stability of insulin. Taken together, our results indicate that PF127 can be potentially used for developing protein delivery system.

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