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PREPARATION OF INSULIN-PLGA IMPLANT AND ITS EFFICACY STUDY

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ABSTRACT

To prepare Insulin-PLGA implants and to investigate the drug release profiles. The implant tablets were prepared using PLGA as carrier and CMC-Na or HA as pore forming agent. The tablets were investigated in vivo and in vitro. SEM showed there were many discontinuous pores in the cross-section of the implants, the release of PLGA/HA implants was rapid, while PLGA/CMC-Na implants released slowly, and the hypoglycemic effect of PLGA/CMC-Na implants at the ratio of 2.5:1 was more than 108 h. Insulin-PLGA implants have good hypoglycemic effects, and can be used as a promising sustained-release preparation for insulin delivery.

KEYWORDS: Insulin microsphere; Implant; Chitosan; PLGA; Blood glucose; Determination; Sustained release.

INTRODUCTION

Insulin has been playing an important role in the treatment of diabetes since its discovery in 1921.[1,2] Insulin is a double-stranded polypeptide hormone consisting of 51 amino acids, which is easily inactivated in the gastrointestinal tract.[3,4] The implant is one of controlled release preparations which can be implanted in vivo or subcutaneously injected by surgery.[5-8] It can avoid the effects of gastrointestinal factors and the first pass effect of the liver.[9,10] Furthermore, stable blood glucose levels can maintain by controlling the release rate of insulin.[10-12]

In this study, insulin implants were prepared with hydrophobic PLGA as a drug carrier, water-soluble HA and CMC-Na as pore forming agents, the preparation method is simple, the formulation of implants and pharmacodynamic analysis in diabetic rats, and in vitro dissolution were investigated.

MATERIALS AND METHODS

Materials

Insulin was purchased from Xuzhou Wanbang jinqiao Pharmaceutical Co, Ltd. (Jiangsu, China). Poloxamer 407 (P407) and Poloxamer 188 (P188) were purchased from BASF (Shanghai, China). Streptozotocin (STZ), citric acid and sodium citrate were purchased from Sigma. Blood glucose monitoring kit was obtained from Great Wall Clinical Reagents Co, Ltd. (Daoding, China). Lantus® (Insulin Glargine Injection) was purchased from Sanofi-Aventis Pharmaceutical Co, Ltd. (Beijing, China). All other chemicals were of analytical grade.

Anhydrous sodium sulfate, ethanolamine and acetonitrile were purchased from Tianjin Kemiou Chemical Reagent Co, Ltd. (Tianjin, China). Sodium hyaluronate (HA) was purchased from Huaxi Freda Biomedical Co, Ltd.(Shandong, China). Carboxymethyl cellulose sodium (CMC-Na) was purchased from Runjie Chemical Reagent Co, Ltd. (Shanghai, China). Poly (lactic-co-glycolic acid) (PLGA) was purchased from Evonik Specialty Chemicals Co, Ltd. (Shanghai, China).

Preparation of PLGA implant tablets

First, PLGA was weighed according to a certain ratio, and insulin solution prepared by dissolving insulin in 0.01 mol / L hydrochloric acid to 40 IU / mL. Then insulin solution was added by stirring and dried in an oven at 30 °C. After that, HA / CMC-Na was added to the mixture at a different ratio. The mixture was tableted (with a diameter 6.5 mm) and stored at 4 °C for use.

Scanning electron microscopy (SEM)

The rats were anesthetized by 10% chloral hydrate solution, the prepared tablets were implanted in rats, and after 48 hours were taken out, the surface morphology of implants before and after implantation was investigated with a model JSM-7500F scanning electron microscopy system (JEOL LTD, Japan) at an accelerating voltage of 10 kV.

Dissolution study

The dissolution behaviors of implanted tablets were investigated. The tablets were placed into 3 mL of pH 7.4 phosphate buffer medium, respectively. The release was carried out in a shaker at a temperature of 37 ± 0.5 °C and a rotation speed of 70 rpm. The media were withdrawn at appropriate time intervals and filtered by 0.45μm filter and replaced with an equal amount of fresh medium then samples were analyzed by HPLC. Each formulation was measured in triplicate.

Preparation of STZ-induced diabetic rats

Healthy male Sprague-Dawley(SD) rats were supplied from the laboratory animal center of Hebei Medical University. The healthy SD rats were fasted for more than 12 h with free access to water. Diabetes was induced by intraperitoneally injected of streptozotocin ($48mg \cdot kg^{-1}$).The rats with blood glucose (BG) greater than 300 $mg \cdot dL^{-1}$ was considered as diabetic rats which was used in hypoglycemic studies in vivo.

The STZ-induced diabetic rats were fasted for 12 h with free access to water and randomly divided into 6 groups. The implanted tablets were subcutaneous injected insulin (10 IU/ kg). Blood samples were collected in tubes containing heparin at various intervals after administration. Then samples were centrifuged after collection and stored at -20℃ for analysis. The BG level was measured using a BG monitoring kit according to the manufacturer's instruction.

RESULTS AND DISCUSSIONS

Preparation of PLGA Implant Tablets

PLGA implant tablets prepared by tabletting with a diameter 6.5 mm. The prepared implant has a complete appearance, smooth and constant weight. Insulin in the tablets is encapsulated in PLGA and insulin released with the degradation of PLGA.

Dissolution study

The amount of insulin release was expressed as the cumulative release.The dissolution curve was shown in Fig.1 and Fig.2.

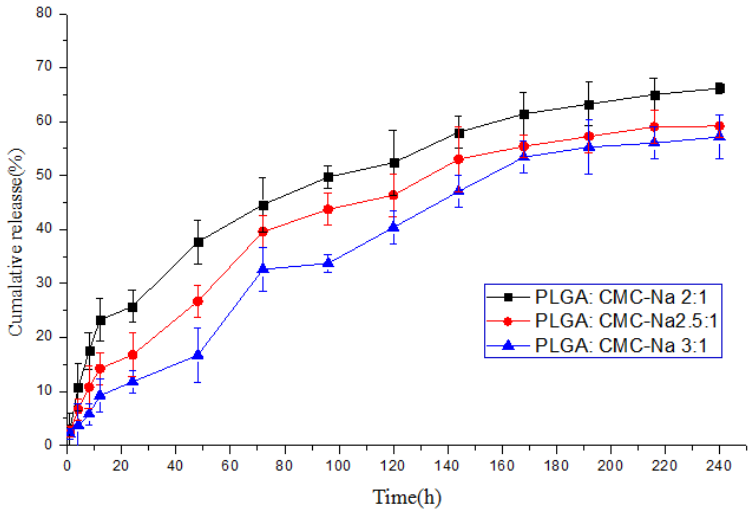

Fig. 1: *In vitro* dissolving curve of PLGA-CMC-Na.

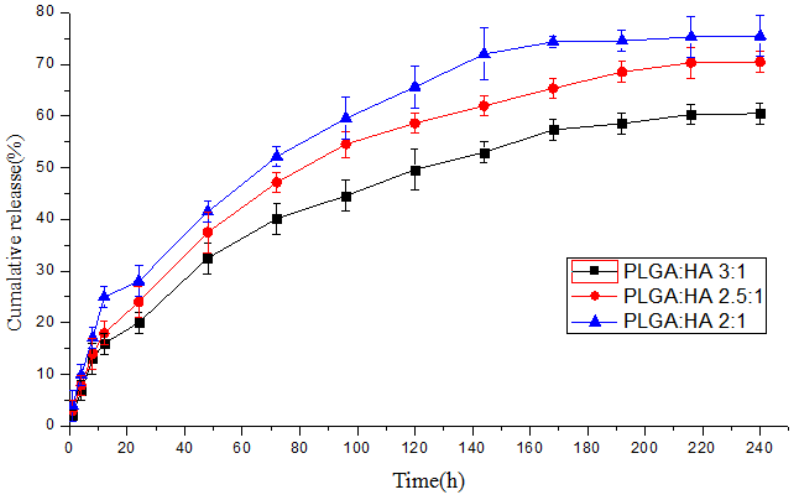

Fig. 2: *In vitro* dissolving curve of PLGA-HA.

As the release time increased, the cumulative release of insulin increased. As can be seen in the Fig.2, the CMC-Na or HA ratio of the implants is smaller when the drug loading is the same and the ratio of PLGA to water-soluble substances is different, the cumulative release of insulin increases faster. The release of PLGA/CMC-Na was the slowest at 3:1. PLGA/CMC-Na 2:1 releases the fastest. The same phenomenon also happened in PLGA/HA tablets. Therefore, the CMC-Na or HA content of the implants has a significant impact on the release of the implants. If the diffusion of insulin is a rate-limited step, an increase in the amount of water-soluble material will result in an increase of surface area and accelerated drug diffusion from PLGA and the hydrolysis of CMC-Na/HA is beneficial to help water enter tablet, then insulin dissolution was increased. Therefore, for implants with a larger

PLGA/CMC-Na or HA ratio, it takes more time for insulin to be released into the media. PLGA/HA had a faster dissolution rate because HA in PLGA/HA swelled quickly on the tablet surface to form a gel and promote the release of drug. For example, CMC-Na / HA at 2:1 showed 66% and 75% release of insulin at 240 h, but the release of insulin was higher at 24 h, reaching 14% and 17%, respectively.

Scanning electron microscopy (SEM)

In SEM study, tablets without implantation were chosen as control. As can be seen from Fig.3, the cross-sectional structure of implant after release is almost completely different from that before release. The surface and cross-section of the implants were densified without holes. However, after 3 days of in vivo implantation, significant changes in the cross-section of the implant were observed and some holes were formed, it was due to the dissolution of CMC-Na or HA and these holes are left. Taking PLGA/CMC-Na 2.5:1 and PLGA/CMC-Na 3:1 as an example, it was shown that the more CMC-Na was contained in the implants, the more holes of the tablets left.

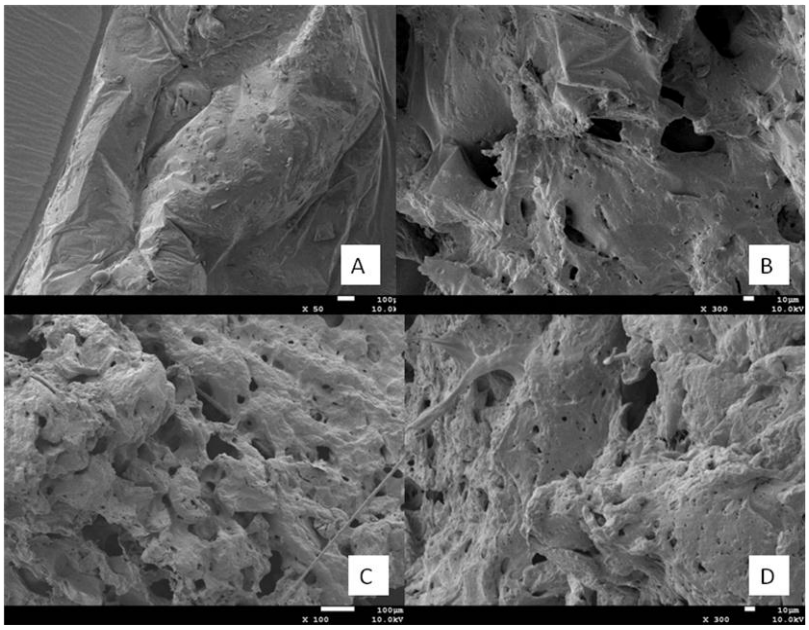

Fig. 3: The electronic micrograph : A: control: B: PLGA-HA 3: 1; C: PLGA-CMC-Na 2.5: 1; D: PLGA-CMC-Na 3:1.

Pharmacodynamic studies in rats

The hypoglycemic effect of insulin implant tablets were evaluated in the study, after implantation, the hypoglycemic effect of insulin tablets investigated and compared with those of physiological saline. No hypoglycemic effect was observed after subcutaneously injection

of saline (Fig.4A). The BG levels were maintained in the hyperglycemia range during the whole experimental time.

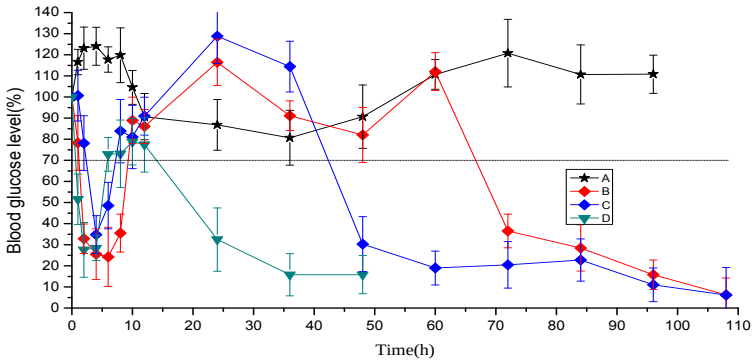

Fig.4. Blood glucose level–time curve : A: Black; B: PLGA: CMC-Na 3:1; C: PLGA: HA 2.5: 1D: PLGA: CMC-Na 2:1）.

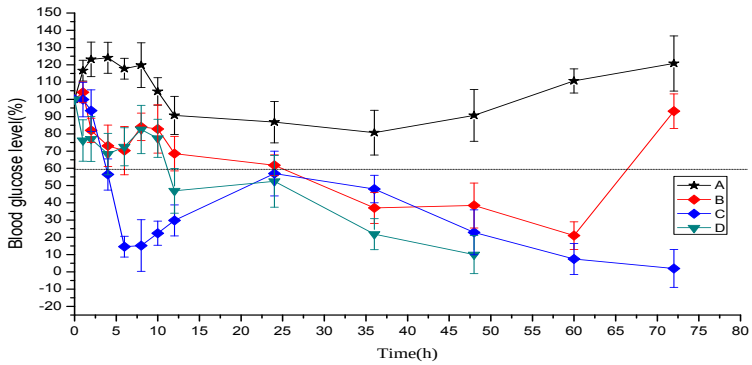

Fig.5. Blood glucose level–time curve : （A:Black; B:PLGA:HA 3:1; C:PLGA:HA 2.5:1; D:PLGA:HA 2:1）.

As can be seen from Fig.4 and Fig.5, the effect of PLGA implant on the rate of release of insulin was directly reflected by the hypoglycemic durations in diabetic rats. Fig.4 showed the effect of CMC-Na amount on the hypoglycemic duration of insulin in diabetic rats. It was found that with the increase of CMC-Na, the volume of the tablet expands and the surface area is increased. CMC-Na is widely used in pharmaceutical formulations as a viscosity-increasing agent with higher concentration used in gel formulation. In the study, this muco-adhesive property is used in implanted tablets designed to prevent insulin diffusion, and modify the release kinetics of insulin.

When the content of PLGA: CMC-Na was 3:1, the insulin release after 72 h of implantation because the content of CMC-Na decreased resulted in slow dissolution rate of insulin. When the content of PLGA: CMC-Na was 2.5:1, the BG level of the insulin treated group showed a rapid decrease at 1 h in the study and the BG level rose to the initial level after 12 hours, BG level began to decrease slowly after 24 hours, returned to the normal level 48 hours later, the hypoglycemic effect could lasted for over 108 hours, the reason for the decrease is due to the degradation of PLGA and the dissolution of CMC-Na. When the PLGA:CMC-Na content was 2:1, the rats died at 48 h, the rapid dissolution of CMC-Na in the body fluid can make the tablet fragment and results in burst release of insulin,then the rats produce a heavy hypoglycemialeading to death.

As shown in Fig.5, the BG in rats treated with PLGA-HA insulin tablets was investigated. There was no significant difference in hyperglycemic effect among different PLGA-HA tablets. The dissolution rate of HA was relative slow compared with that of CMC-Na, so the amount of HA did not play an important influence on the release of insulin. In PLGA-HA tablets, HA could dissolve in the body fluid slowly to form gel wrapped around the tablets.
When the content of PLGA/HA was 3:1, after 62 hours the BG began to rise and recovered to hyperglycemic state at 72 h. When the content of PLGA/HA was 2.5: 1,the BG rapidly decreased within 6 h, increased from 8 h to 24 h. When the PLGA/HA content was 2:1, the rats died at 48 h.

CONCLUSIONS

In this paper, PLGA implant were prepared and the effect of the ratio of implant to CMC-Na / HA, CMC-Na / HA on the release of insulin were investigated. The prepared implant has a smooth and compact surface. The in vitro release test of implants was carried out, and the structure and pharmacodynamics of implants were further investigated.The results showed that SEM shows there were many discontinuous pores in the cross-section of the implants, the release of PLGA-HAwasmore rapid than that of PLGA/CMC-Na implants, and when the ratio of PLGA to CMC-Na is 2.5:1, the hypoglycemic effect is over 108 h.

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DECLARATION OF INTEREST

The authors report no declarations of interest.

REFERENCES

1. Nielsen E J B, Yoshida S, Kamei N, et al. In vivo, proof of concept of oral insulin delivery based on a co-administration strategy with the cell-penetrating peptide penetratin. *Journal of Controlled Release*, 2014; 189(36): 19.
2. Tyagi P. Insulin delivery systems: Present trends and the future direction. *Indian Journal of Pharmacology*, 2002; 1(6).
3. Heinemann L, Pfützner A, Heise T. Alternative routes of administration as an approach to improve insulin therapy: update on dermal, oral, nasal and pulmonary insulin delivery.*Current Pharmaceutical Design*, 2001; 7(7): 1327-1351.
4. El-S K, Morishita M, Onuki Y, et al. Current challenges in non-invasive insulin delivery systems:a comparative review. *Adv Drug Deliv Rev*, 2007; 59(15): 1521-1546.
5. Lin Ying, Zhu Dequan, Tan Fengping, et al. Progress in implantable drug delivery systems. *Fine chemical engineering*, 2005; 22(1): 32-35.
6. Wang F, Lee T, Wang C H. PEG modulated release of etanidazole from implantable PLGA/PDLA discs. *Biomaterials*, 2002; 23(17): 3555-3566.
7. Naraharisetti P K, Ong B Y S, Xie J W, et al. In vivo performance of implantable biodegradable preparations delivering Paclitaxel and Etanidazole for the treatment of glioma.*Biomaterials*, 2007; 28(28): 886-894.
8. Rahman C V, Kuhn G, White L J, et al. PLGA/PEG-hydrogel composite scaffolds with controllable mechanical properties. *Journal of Biomedical Materials Research Part B Applied Biomaterials*, 2013; 101B(4): 648.
9. Uhrich K E, Cannizzaro S M, Langer R S, et al. Polymeric systems for controlled drug release. *Chemical Reviews*, 1999; 99(11): 3181-3198.
10. Gupta K C, Ravi Kumar M N. Drug release behavior of beads and microgranules of chitosan. *Biomaterials*, 2000; 21(11): 1115-1119.
11. LU B. New Techniques and New Dosage Froms of Drugs. Beijing：People′s Medicine Publishing House，1998; 503-515.
12. Liu Huagang, Lu quartz. Research progress of implantable sustained release drug delivery system. *Modern medicine and health*, 2005; 21(2): 157-159.