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PREPARATION AND PHARMACODYNAMICS STUDY OF INSULIN IMPLANT TABLETS

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ABSTRACT

In this study, insulin implant tablets was prepared and its pharmacodynamics was studied in rats. Polylactic acid (PLA) and poloxamers were used as carriers to prepare insulin implanted tablets. In vivo pharmacodynamics of insulin-implanted tablets in rats was investigated. Glucose oxidase method was used to measure blood glucose concentration. The in vitro release of implants was influenced by the amount of Polylactic acid and poloxamer, the cumulative release of insulin was 81.9 ± 1.3% ~ 90.4 ± 1.5% after 36 hours. In vivo pharmacodynamics results showed that insulin implant tablets had a sustained release effect up to 84 h. The quality of insulin implants is easy to control, and the tablets can produce a sustained release effect. It can provide a convenient and effective way for diabetic patients.

KEYWORDS: Diabetes; insulin; PLA; implant; pharmacodynamics.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and is caused by impaired insulin secretion or impaired biological function. Long-term hyperglycemic state can lead to chronic injury or dysfunction in the eye, kidney, heart, blood vessels, nerves and other tissues of diabetic patients.[1] [2] In recent years, the number of people suffering from diabetes is increasing, diabetes has become diseases that cause serious harm to human health.

Insulin is the only hormone that lowers blood sugar in the body. In clinical practice, insulin is mainly administered subcutaneously. However, patients with diabetes need multiple injections a day and long-term frequent injections can cause psychological and physical pain to patients. Therefore, the need to find alternative insulin delivery system has become a hot spot in the insulin delivery study.

Oral mucosa[3], nasal[4], pulmonary[5], transdermal[6] and oral[7] administration of insulin, has been reported in the literature. However, since insulin is a biological macromolecular drug, the drug permeability by oral, nasal and transdermal methods is not ideal, oral bioavailability is low because insulin is susceptible to pepsin hydrolysis, although pulmonary administration is effected[5], its safety needs to be further verified, the study found that implanting insulin into tablets is an hopeful effective method.[8] Implant tablets can be immune to insulin from the interference of the liver first pass effect, reducing its blood concentration fluctuations, extending its half-life in vivo, at the same time, the implant tablets with controlled release can eliminate the uneven dosage and intermittent drug delivery caused by the peak and valley phenomenon.

As a biodegradable polymer material, polylactic acid (PLA) has excellent biocompatibility and good physical properties. After being degraded, it produces carbon dioxide and water without any harm to the human body[9], so PLA can be used for drug delivery, wound dressing and many other uses. The US FDA has approved it as a medical material for the preparation of controlled release drugs.[10]

In this experiment, insulin tablets were prepared using PLA-poloxamer as a carrier. The impact of formulation factors on insulin release in vitro were investigated, the study was aimed to prepared the insulin tablets with sustained release, it will provide a convenient and effective administration way for diabetic patients.

MATERIALS AND METHODS

Instruments

DHG-9070 heated oven (Zhengzhou Great Wall Branch Co., Ltd.); single-punch tablet machine (Shanghai Tian Fan medicine factory); THA-82 gas bath temperature oscillator (Jintan Medical Instrument Factory) LC-20A Shimadzu high performance liquid chromatography (Shimadzu International Trading (Shanghai) Co., Ltd.).

MATERIALS

Insulin was purchased from Xuzhou Wanbangjinqiao Pharmaceutical Co, Ltd (Jiangsu, China). Poloxamer 407 (P407) and Poloxamer 188 (P188) were purchased from BASF(Shanghai, China). Streptozotocin (STZ) was purchased from Sigma. Citric acid and sodium citrate were purchased from Beijing Chemical Reagent Company. PLA was purchased from Evonik Specialty Chemicals Co, Ltd.(Shanghai, China).; Chloral hydrate was purchased from Tianjin Guangfu Fine Chemical Research Institute; Glucose (Analytical Pure) was obtained from Great Wall Clinical Reagents Co, Ltd.(Daoding, China).

Experimental animals

Male SD rats, 200 ± 20 g, provided by Hebei Medical University Experimental Center.

Preparation of insulin implant tablets

Insulin implant tablets were prepared as follows: a certain amount of PLA, P188 and P407 were accurately weighed, and PLA and P188 or PLA and P407 at the ratio of 2: 1, 3: 1, 4: 1 were mixed well and granulated after the addition of insulin solution. The wet granules were placed in a oven and dried at 30°C for 3 hours. The whole granules were selected and tableted. The size of the insulin tablets was 2 IU per tablet.

Insulin tablet quality evaluation

Hardness

The hardness of implant tablets was measured with the use of tablet hardness tester, Experimental results in Tab. 4.

Determination of content

10 implant tablets were accurately weighed and crushed and a certain amount of powder (about 2 IU insulin) was placed in 10mL volumetric flask, then hydrochloric acid solution (pH=2) was added to the whole volume, after filtration through a 0.45μm membrane filter, the drug content was determined by HPLC using a Venusil XBP-C18 column (250mm × 4.6mm, 5μm). The mobile phase was consisted of phosphate buffer (pH 2.3) and acetonitrile (74:26). The column temperature was room temperature. The UV detection wavelength was 214 nm. The flow rate was 1.0 ml / min and the injection volume was 20μL.

In vitro release test

The implant tablet was taken into a 5mL EP tube, adding with 2mL phosphate buffer (pH 7.4) and placed in a thermostatic shaker at a set temperature of (37±0.5) °C with an oscillation speed of 90 rpm. The release media was removed at predetermined time intevals and the concentration of the drug was detected by HPLC mentioned above.

Scanning electron microscopy(SEM)

Insulin implanted tablets were taken from the rats and dried, then fixed with a double-sided conductive adhesive and sprayed under vacuum, 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.

Glucose oxidase method for the determination of blood glucose

The detection of glucose oxidase (GOD) method is as follows

$$\text{glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{4-aminoantipyrine} + \text{phenol} \xrightarrow{\text{CAT}} \text{Red quinone compounds} + 2H_2O + O_2$$

Linear inspection

The appropriate amount of glucose stock solution was taken, adding with deionized water to prepare the concentration of 0.56-44.40mmol/L of glucose solution, 10μL of different glucose solution was mixed with 1.0mL working solution respectively in 37 ° C water bath for 10 minutes, the absorbance was detected at 505 nm.

Repeatability and Recovery

The concentration of 27.75mmol/L glucose solution was repeatly measured for 6 times under the test conditions. For recovery determination, 10μL of glucose standard solution was added to the rat serum, measured to calculate the recovery rate.

Stability

The glucose standard and working solution mixed at 37 ℃ in water bath, continuously sampling within one hour to measure its absorbance at 505 nm to investigate the stability of the sample.

Pharmacodynamics of insulin implant tablets

Type II diabetic rat model

Healthy male Sprague - Dawley rats were fasted for 12 hours and were free to water. Rats were injected with 10% STZ citrate buffer at a dose of 48 mg • kg-1, blood glucose was measured after 7 days, and the rats with blood glucose more than 16.67mmol / L was used for the in vivo of pharmacodynamic study.

Thirty diabetic rats were divided into six groups and fasting for 12 hours before experiment. The body weight was weighed and recorded, 10% chloral hydrate solution was intraperitoneally injected at the dose of 3 ml / kg. Insulin implants were subcutaneously implanted at a dose of 10 IU/kg. After administration, 0.3mL of blood was collected from the retro-orbital venous plexus at a set time interval. After centrifugation, the upper plasma was taken and blood glucose was measured by the GOD method.

RESULTS AND DISCUSSION

Linear inspection

The linear regression equation was: Y = 0.0554X-0.0105, R2 = 0.9997. When the concentration was within 0.56~44.40mmol/L, the linear relationship between glucose concentration and absorbance was good. The experimental results were shown in Tab.1.

Tab.1: Absorbance at different sample concentrations

C(mmol/L)	0.56	1.11	2.22	2.78	5.55	13.88	27.75	44.40
A	0.022	0.058	0.126	0.152	0.295	0.726	1.517	2.467

Repeatability and Recovery

The recoveries of this method ranged from 99.3% to 101.5%, indicating that the recoveries of low, medium and high blood glucose levels were all good and the RSD value did not exceed 2.0%, indicating that the recovery meet the methodological requirements. The experimental results were shown in Tab.2.

Tab.2 Result of the determination of recovery (n = 3)

Add concentration (mmol/L)	Recover concentration (mmol/L)	Recovery rate (%)	RSD (%)
1.11	1.12	100.9	1.13
3.33	3.38	101.5	
5.55	5.51	99.3	

Stability

The experimental results were shown in Tab.3. As can be seen from Tab.3,there was no significant change in the absorbance value of the sample in the water bath within 1 hour and the stability of the sample was good.

Tab.3: Results of stability experiment.

Time(min)	10	20	30	40	50	60	RSD(%)
Absorbance	0.527	0.534	0.529	0.528	0.530	0.529	0.46

Hardness

The hardness of the implants ranged from 23.6 N to 31.3 N.

Tab.4 The hardness of implants

Formulation	PLA/P188 2:1	PLA/P188 3:1	PLA/P188 4:1	PLA/P407 2:1	PLA/P407 3:1	PLA/P407 4:1
Hardness（N）	25.5±1.9	26.5±1.9	27.7±3.6	26.8±3.1	26.5±4.5	29.7±1.6

Release test

As can be seen in the Fig.1, the cumulative release of six formulations was in the range of (70.6 ± 1.8)% ~ (84.8 ± 1.6)% after 12 hours. The release rate of PLA / P407(2: 1) > PLA / P407 (3: 1)> PLA / P188 (2: 1)> PLA / P188 (3: 1)> PLA /P407 (4: 1)> PLA / P188 (4: 1), the ratio of poloxamer to PLA and the type of poloxamer influenced the release rate of the implant tablets.

P188 and P407 were water-soluble excipients, can help to increase the dissolution rate of insulin in tablets, improving the proportion of poloxamer in implants tablets, can speed up the release of drugs. The insulin release of implants tablets was mainly dominated by diffusion, insulin in the surface of the implant tablets diffused into the release medium, the internal dissolved insulin penetrated the PLA matrix from the pores of poloxamer. After 12 hours, the drug release rate slowed down. The dissolution and degradation rate of PLA determined the release rate of insulin. After 36 hours release, the cumulative release of 6 formulations was 81.9 ± 1.3% ~ 90.4 ± 1.5%.

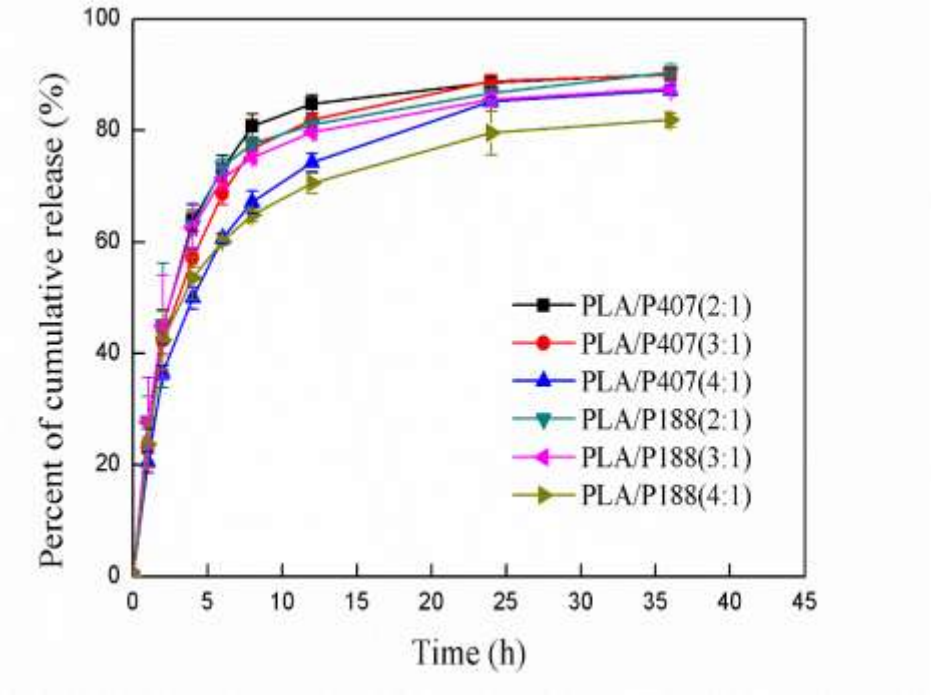
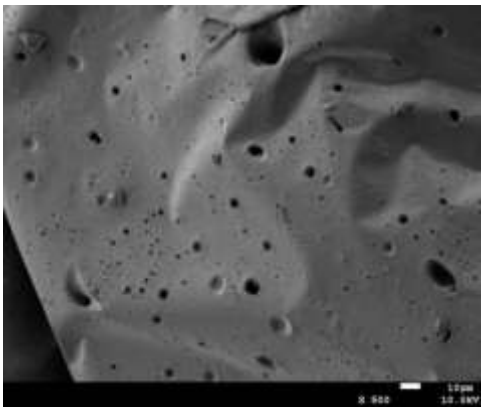


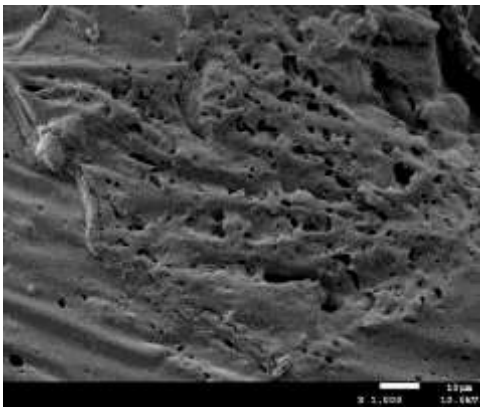
Fig.1 The in vitro release experiment of insulin-loaded PLA implanted tablets.

SEM results

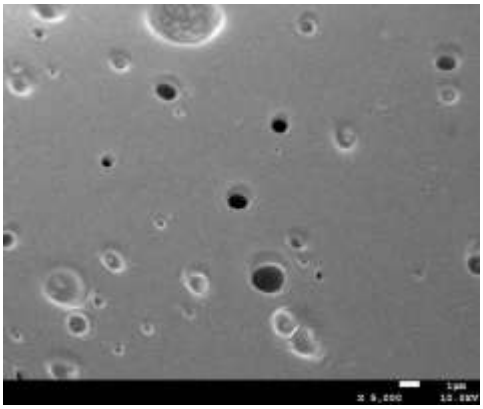
From the SEM, the surface of the PLA implants was smooth after implanted 3 days in vivo, and the surface and interior of the implants were covered with micropore-like structures with different pore sizes. This was due to the formation of tiny pores in the implanted tablets after dissolution of P188 and P407.



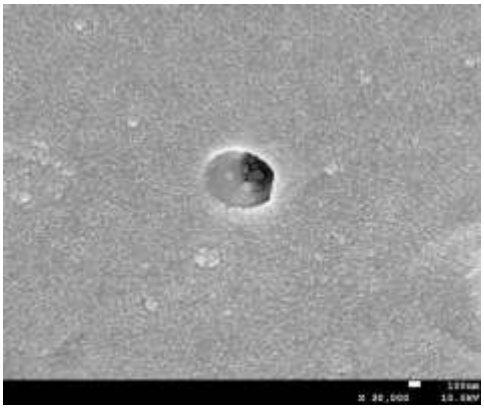
A Magnified 500 times SEM results



B Magnified 1000 times SEM results



C Magnified 5000 times SEM results



D Magnified 30,000 times SEM results

Fig. 2 SEM of PLA implanted tablets after dissolution for 3 days

Pharmacodynamic results

The results have shown that PLA and poloxamer can be selected as sustained-release materials.

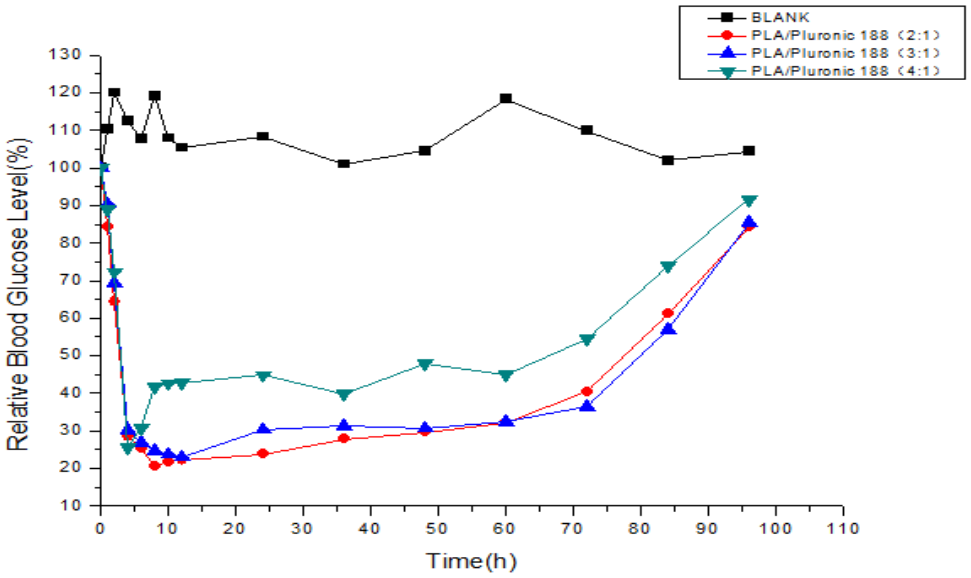


Fig. 3 Plasma glucose level after subcutaneous administration of various insulin tablets.

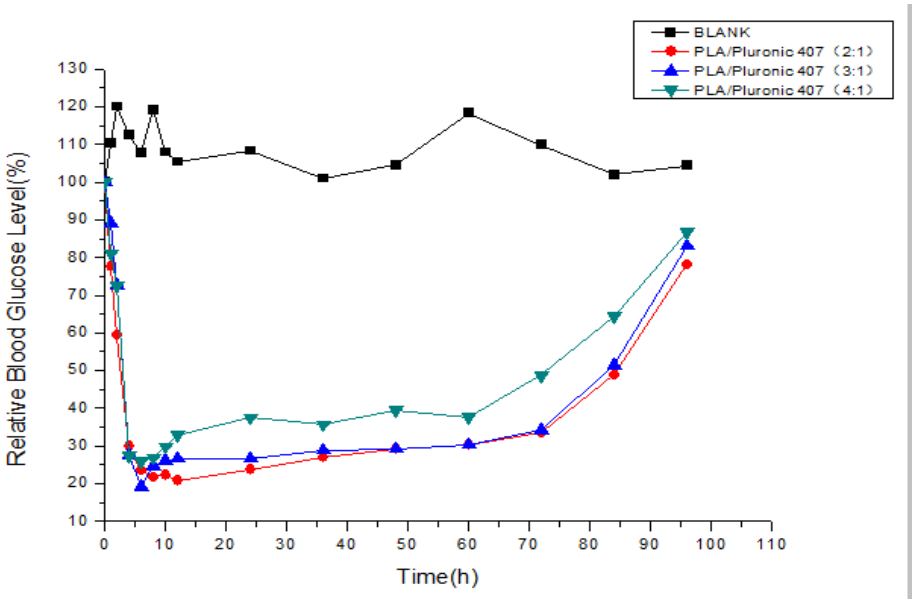


Fig. 4 Plasma glucose level after subcutaneous administration of various insulin tablets.

As shown in Fig. 3 and Fig. 4, PLA and poloxamer were selected as sustained-release materials, and the rate of insulin release was changed by changing the ratio of the two materials in the insulin-implanted tablet. When PLA used as a sustained release material alone, insulin was almost not released and the body hypoglycemic effect was poor. As the proportion of poloxamers increased, the hydrophilicity of the implanted tablet increased, accelerating the chance of insulin entering body fluids, so the hypoglycemic effect of polylactic acid / poloxamer (2: 1) was significantly better than that of PLA / poloxamer (4: 1). Most of the poloxamers in the first 72 hours had been dissolved completely, PLA dissolved slowly and the amount of insulin decreased at the same time, and the blood glucose began to increase slowly. After 84 hours, it began to enter the high blood sugar level (16.67mmol / L), so the optimal formulation was determined as PLA / poloxamer (2: 1) and the effective duration of the implanted tablets was about 84 hours.

CONCLUSION

In this study, PLA-poloxamer was used as carriers to prepare insulin implants. The pharmacodynamic studies showed that insulin implants had a good hypoglycemic effect in rats. Due to the water-solubility of poloxamer, it can increase the hydrophilicity of the polymer and form a water-soluble channel inside the implanted tablet, which was beneficial to the release of the drug.[11] In vitro experiments showed that increasing the hydrophilicity of the polymer can improve the cumulative release of drug. The insulin implant tablets prepared in this study can achieve sustained and slow drug release, both in vitro and in vivo have a

good sustained-release effect, which can meet the need of diabetes treatment and provide a new way for the treatment of diabetes.

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DECLARATION OF INTEREST

The authors report no declarations of interest.

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