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COMMUNICATION

Preparation and Evaluation of Insulin-Loaded Polylactide Microspheres Using Factorial Design

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ABSTRACT

The aim of this work was to study the influence of the concentration and molecular weight of poly(DL-lactide) (PLA) on the characteristics and in vivo biological activity of protein-loaded microspheres. At the same time, an attempt was made to achieve further optimization of the formulation. In the study, insulin was chosen as a model of protein drugs. Nine formulations of injectable insulin-loaded PLA microspheres were prepared using an emulsification and solvent evaporation process according to a factorial design. The trapping efficiency, drug loading, and the drop percentages of blood glucose levels at 24 hr and 72 hr in mice were used to evaluate the formulations. The results showed that PLA molecular weight and, especially, PLA concentration exerted influences on the characteristics and in vivo biological activity of insulin-loaded microspheres. The drug-trapping efficiency increased with the increase of the polymer concentration. The drug loading decreased with the increase of the polymer concentration and was not obviously affected by PLA molecular weight. The drop percentage of blood glucose level at 24 hr increased with the increase of polymer concentration and molecular weight. At 72 hr, the drop percentages of blood glucose levels were slightly increased with the increase of PLA concentration and then significantly decreased after the PLA concentration was above 150 mg/ml. An optimized formulation was prepared with PLA-

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10k at a concentration of 200 mg/ml. The experimental values of the response variables were close to the predicted values. The results suggest that the in vivo release behavior should be taken into consideration in the design of protein-loaded PLA microspheres.
Key Words: *Insulin; Microspheres; PLA.*

INTRODUCTION

The delivery of protein and polypeptide drugs for therapeutic purposes presents a major challenge to pharmaceutical scientists since several problems are associated with the therapeutic effectiveness of the drugs, such as a very short physiological half-life and very poor membrane transport, accompanied by extensive metabolism. Recently, biodegradable microspheres containing peptides and proteins have received much attention for overcoming these problems (1–3). Many studies on the preparation and formulation of protein-loaded microspheres have been reported (4–6). However, the formulations of protein-loaded microspheres are far from being complete. In most of the studies, only trapping efficiency or in vitro release plots were used for the optimization of formulations. The in vivo biological activity of proteins was hardly taken into consideration in the evaluation of formulations.

In the present paper, insulin was chosen as a model of protein drugs since it is one of the most widely used protein drugs and has a short half-life. Nine formulations of injectable insulin-loaded poly(DL-lactide) (PLA) microspheres were prepared using an emulsification and solvent evaporation process according to a factorial design (7). The aim of this work was to study the influence of the concentration and molecular weight of PLA on the characteristics and in vivo biological activity of the formulations. At the same time, an attempt was made to achieve further optimization of the formulation. Besides trapping efficiency and drug loading, the drop percentages of blood glucose levels at 24 hr and 72 hr in mice were used to evaluate the formulations.

EXPERIMENTAL

Materials and Methods

PLA (average molecular weight 10K, 15K, 20K; batches 940322, 940302, 940328) was supplied by the Institute of Shang Dong Medical Instruments (China). Porcine insulin (26.4 IU/mg; batch 950102) was purchased from Xu Zhou Biochemical Company (China). All other chemicals were analytical grade and were used as supplied. Chromatographic solvents were high-performance liquid chromatography (HPLC) grade.

Preparation of Insulin-Loaded PLA Microspheres

PLA microspheres containing insulin were prepared by an emulsification and solvent evaporation technique reported by Wada et al. (8). The only difference is that we used Span-80 (0.15%) as an emulsifier instead of soybean lecithin. The polymer concentration was regulated by changing the weight of PLA dissolved in a fixed volume of the solution. The drug-trapping efficiency was calculated by referring the percentage of encapsulated insulin to the total insulin used to prepare the microspheres.

The drug entrapped inside the microspheres was detected by HPLC (9).

In Vivo Experiments

Diabetes was induced in female ICR mice (body weight approximately 22 ± 2 g) by intravenous injection of streptozotocin (Sigma Chemical Co., St. Louis, MO; in pH 4.5, 0.1 M citrate buffer) at a dose of 50 mg/kg body weight. Five mice were left as normal controls. After ensuring the induction of diabetes by measuring the blood glucose level compared with that of the normal control, five mice received the blank microspheres (5 mg s.c.) as diabetic controls. Mice ($n = 54$) were divided into groups and treated with the insulin-loaded microspheres with different process parameters at a dose of 644 IU/kg. Blood was taken from veins of the eye at scheduled intervals, and the glucose levels were measured using a glucose test kit (Glucose B-Test Kit, Shanghai Medicine Chemistry Institute, China). The mice were fasted for 12 hr before blood samples were collected.

RESULTS AND DISCUSSION

Optimization of Microsphere Formulation

Nine formulations of microspheres were produced to elucidate the effects of concentration and molecular weight of PLA. The experimental design and results are

Table 1

Results of Nine Formulations of Insulin-Loaded PLA Microspheres Prepared Using an Emulsification and Solvent Evaporation Process

MW (K)	Concentration of PLA (mg/ml)	Trapping Efficiency ($n = 3$)	Content ($n = 3$)	D_{24} ($n = 5$)	D_{72} ($n = 5$)
10	100	51.03% ± 3.51	10.04% ± 1.24	39.07% ± 14.79[a]	70.75% ± 15.64[a]
10	200	80.98% ± 2.88	8.19% ± 0.21	68.11% ± 11.50[a]	67.97% ± 13.50[a]
10	300	77.47% ± 3.18	5.29% ± 0.33	66.18% ± 13.16[a]	3.72% ± 16.14[b]
15	100	38.51% ± 2.27	11.45% ± 0.50	7.48% ± 6.37[b]	8.78% ± 1.26[b]
15	200	45.06% ± 1.96	4.57% ± 0.65	19.55% ± 19.27[a]	31.30% ± 6.99[a]
15	300	62.81% ± 1.76	5.03% ± 0.07	54.04% ± 9.64[a]	71.17% ± 8.63[a]
20	100	57.84% ± 5.13	12.21% ± 0.04	30.17% ± 11.84[a]	52.39% ± 16.84[a]
20	200	57.82% ± 1.75	6.78% ± 0.28	22.50% ± 4.04[a]	53.14% ± 9.80[a]
20	300	61.09% ± 5.24	4.56% ± 0.65	14.29% ± 9.62[a]	63.49% ± 5.4[a]

D_{24} and D_{72} indicate the drop percentages of blood glucose levels at 24 hr and 72 hr.
[a] $p < .05$.
[b] $p > .05$ vs. the control group (by paired t test with SigmaPlot computer software).

summarized in Table 1. To compare the formulations quantitatively, the data were fitted using Sigmaplot 5.0 software with the equations

$$T = 176.9629 - 19.8035x + 0.2164y + 0.6237x^2 - 0.0003y^2 \qquad R = 0.876 \tag{1}$$

$$C = 26.0028 - 0.9923x - 0.0947y + 0.0331x^2 + 0.0002y^2 \qquad R = 0.944 \tag{2}$$

$$D_{24} = 7.6962 + 0.7696x + 0.0945y + 0.0770x^2 + 0.0007y^2 \qquad R = 1 \tag{3}$$

$$D_{72} = 154.7277 - 20.4822x + 0.8942y + 0.6215x^2 - 0.0031y^2 \qquad R = 1 \tag{4}$$

where x is molecular weight, y is polymer concentration, T is insulin trapping efficiency, C is insulin content, and D_{24} and D_{72} are as noted in Table 1.

PLA-10K at a concentration of 200 mg/ml was chosen as an optimal formulation according to the results of the evaluations. Six batches of the optimized formulation were prepared. The experimental values were relatively close to those predicted by the derived equations (Table 2). In fact, the trapping efficiency could be increased further if a siliconized container was used in preparation.

In addition, the size distribution of the microspheres was determined by means of an optical microscope. However, only small differences in particle size among the different formulations were observed. The mean diameter of the microspheres was about 20 μm.

Effects of PLA Concentration and Molecular Weight

The drug-trapping efficiency increased with the increase of the polymer concentration. The reason could be contributed to (*a*) high mass transfer resistance with the more viscous medium because of the increase of the PLA concentration or (*b*) the high PLA precipitation rate. When more polymer is present in the medium, the free volume available for it is reduced. Therefore, the possible conformations that the PLA can adopt decrease, whereas precipitation occurs more readily (10). The mean drug-trapping efficiency decreased as the polymer molecular weight changed from 10K to 15K and increased from 15K to 20K. By comparison, the maximum trapping efficiency was achieved by PLA-10K.

Table 2

Predicted and Observed Values of Insulin-Loaded Microspheres with PLA-10K at Concentration of 2000 mg/ml

Indices	Predicted Values (%)	Observed Values (%)
Drug content	6.78	7.88 ± 1.65
Trapping efficiency	71.94	75.88 ± 2.03
D_{24}	68.11	72.16 ± 4.15[a]
D_{72}	67.97	48.85 ± 3.79[b]

[a] $p < .01$.
[b] $p < .05$ (vs. the diabetic control).

The drug loading was not obviously affected by polymer molecular weights and decreased with the increase of the polymer concentration. This effect might be related to the use of a higher weight polymer to increase the polymer concentration. The actual insulin entrapment with PLA microspheres decreased (11).

Most of the formulations showed significant effects of lowering blood glucose levels versus the diabetic control (Table 1). Figure 1 was produced according to Eqs. 3 and 4 to illustrate the effect of PLA concentration and molecular weight on the drop percentages of blood glucose level. At 24 hr, the drop percentages of the blood glucose levels increased with the increase of polymer concentration and molecular weight (Fig. 1a). This means that a lower burst release value was produced by low polymer concentration and molecular weight. At 72 hr, the drop percentages of the blood glucose levels were slightly increased with the increase of PLA concentration and then significantly decreased after the PLA concentration was above 150 mg/ml (Fig. 1b). High drop percentages were obtained by the microspheres with PLA-10K at 72 hr. By combination of the data at 24 hr and 72 hr, the sustained in vivo release behavior could been produced by the microspheres with PLA-10k at a concentration range of 100–200 mg/ml. The reason that a sustained action did not happen at a high polymer concentration and high molecular weight is probably because of the combination of three factors: (*a*) heavy accumulation of the protein on the microsphere surface due to rapid precipitation at a high polymer concentration, (*b*) the native conformation change of proteins in microspheres due to the strong interaction between protein and polymer at a high polymer concentration (12,13), and (*c*) an incomplete in vivo protein release resulting from adsorption and aggregation at a high polymer concentration and high polymer molecular weight (14). However, most in vitro experiments supported that reduced burst release and sustained-release behavior could be obtained using microspheres with high polymer content and high molecular weight (15,16).

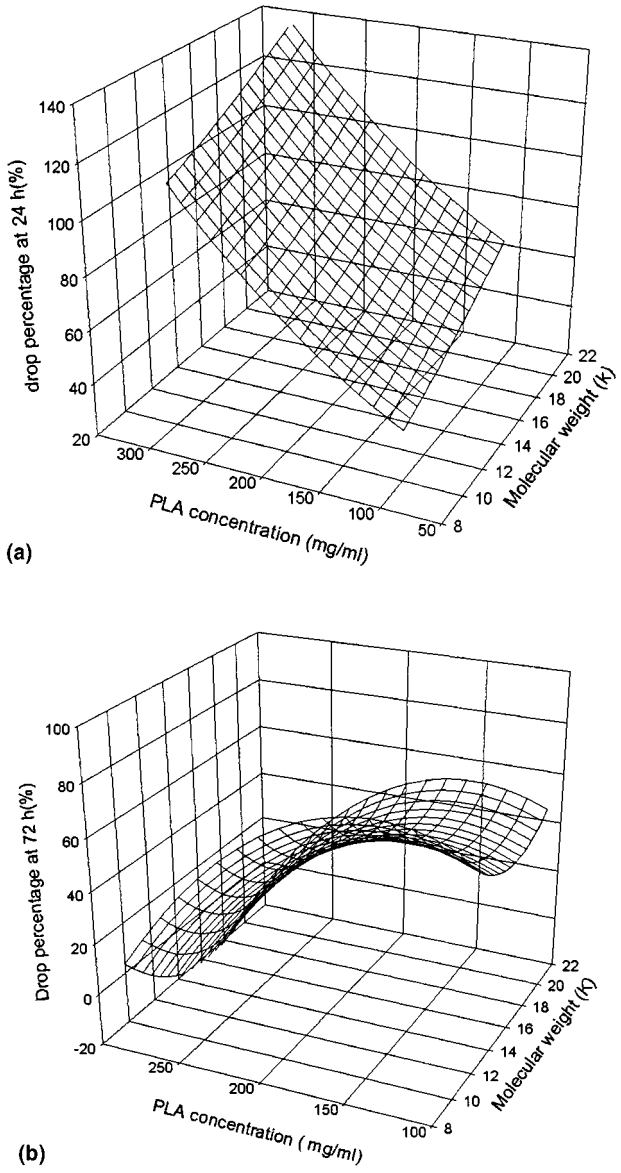

Figure 1. Response surface diagram fro drop percentages of blood glucose levels at (a) 24 hr and (b) 72 hr in mice treated with insulin-loaded PLA microspheres with variation in polymer concentration and molecular weight.

CONCLUSION

PLA molecular weight and, especially, PLA concentration exerted influences on the characteristics and in vivo biological activity of insulin-loaded microspheres, and in vivo release behavior should be taken into consideration in the design of protein-loaded PLA microspheres.

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