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Prolonged hypoglycemic effect of insulin-loaded polybutylcyanoacrylate nanoparticles after pulmonary administration to normal rats

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Abstract

Insulin-loaded polybutylcyanoacrylate nanoparticles were prepared by emulsion polymerization. The mean diameter of the nanoparticles was 254.7 nm with a polydispersity of 0.064. The associating ratio of insulin to the nanoparticles reached 79.1%. Studies on in vitro release kinetics showed that release profiles can be modeled using a biexponential function and the burst effect was obvious. After various doses of insulin-loaded nanoparticles were intratracheally given to normal rats, significant decrease of glucose level was achieved at each dose group from 5 to 20 IU kg^{-1}. The minimum blood glucose concentration reached 46.9%, 30.4% and 13.6% of the initial level after pulmonary delivery of 5, 10 and 20 IU kg^{-1} insulin-loaded nanoparticles to normal rats, respectively. The time to reach the minimum blood glucose level (T_{min}) was 4, 4 and 8 h for three doses, respectively. The duration of glucose level below 80% of insulin-loaded nanoparticles was much longer than that of insulin solution at every dose. Relative pharmacological bioavailability of insulin-loaded nanoparticles by pulmonary administration was 57.2% over the same formulation by subcutaneous administration. © 2001 Elsevier Science B.V.

Keywords: Insulin; Nanoparticles; Hypoglycemic effect; Rats; Pulmonary administration; Polybutylcyanoacrylate

1. Introduction

Attempts have been made to alter the route of administration of insulin in the treatment of insulin-dependent diabetes (Chien and Banga, 1989), ever since the discovery of insulin. Pulmonary delivery of peptides and proteins has attracted increasing attention, because it may become an important new route of noninvasive system administration (Colthorpe et al., 1992; Kobayashi et al., 1994; Edwards et al., 1997). Transpulmonary delivery of peptides and proteins may be expected to have higher rates of systemic absorption than other noninvasive routes, since it provides a large surface area, the alveolar epithelium where absorption take place is thin, and

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enzyme activity is relatively low (Shen et al., 1999).

The controlled release of the therapeutic agent by use of biodegradable polymeric materials may provide better local therapy in lung or systemic therapy (Gonda, 1990). Sustained release in the lung tissue from the therapeutic carrier system, such as liposomes, microspheres or nanoparticles could prolong the residence of an administered drug in the alveoli, resulted in stable and prolonged pharmacological effects. Patient compliance is also expected to increase as dosage frequency is reduced. Pulmonary delivery of insulin-loaded DL-lactide/glycolide copolymer nanospheres (Kawashima et al., 1999) as well as the iodinated nanoparticles for CT enhancement (McIntire et al., 1998) was reported.

In the present study, insulin-loaded polybutylcyanoacrylate nanoparticles were prepared and its basic properties were determined. The prolonged hypoglycemic effect of the nanoparticles after pulmonary administration at different doses to normal rats has been investigated using the insulin solution as the positive control, and the relative pharmacological bioavailability was calculated by comparing the area above the blood glucose curve between pulmonary administration and subcutaneous administration.

2. Materials and methods

2.1. Materials

Crystalline porcine zinc insulin (26.3 IU mg^{-1}) was supplied by Xuzhou biochemical plant (Xuzhou, China), and butylcyanoacrylate was purchased from Southern Medical Glue Co. (Shanzhen, China). Dextran 70 was from Pharmacia (Uppsala, Sweden) and sodium pentobarbital from the China Pharmaceutical Co. Reagents for blood glucose assay were prepared by dissolving 100 $U \cdot mg^{-1}$ glucose oxidase, 1.5 mg peroxidase, 25 mg 4-aminioantipyrine and 250 mg NaN_3 in 250 ml 50 $mmol \cdot l^{-1}$ Tris–HCl buffer (pH 7). All other chemical substances are of analytical grade.

Wistar rats were purchased from the Animal Service of Medical Center, Peking University.

2.2. Preparation of insulin-loaded polybutylcyanoacrylate nanoparticles

The insulin-loaded polybutylcyanoacrylate nanoparticles were prepared by emulsion polymerization (Zhang and Liao, 1996). Simply, 100 mg Dextran 70 was dissolved into 9 ml distilled water (pH 3), then about 100 μl α–butylcyanoacrylate was added, drop by drop, under gentle agitation. After 1 h agitation, 1 ml insulin solution (50 $IU \cdot ml^{-1}$) was added, and the agitation was kept for 2 h under room temperature to obtain 5 $IU \cdot ml^{-1}$ insulin nanoparticle colloid solution. 10 and 20 $IU \cdot ml^{-1}$ insulin nanoparticle colloid solution were prepared by the similar way.

2.3. Determination of the nanoparticle size

The mean size and the polydispersion of the insulin-loaded nanoparticles were determined by dynamic light-scattering (DLS) measurement (Brookhaven Instruments, USA).

2.4. Determination of the associating ratio

The insulin in nanoparticles was analyzed by reversed-phase high performance liquid chromatography method using a model ZY1104 XWG-C_{18} column. The insulin nanoparticle colloid solution was centrifuged at 35,000 r min^{-1} for 30 min, then 25 μl of the supernatant was injected into the column, eluted with acetonitrile and phosphates buffer (pH 3) at a rate of 1.0 $ml \cdot min^{-1}$, and detected by UV spectrometry at 276 nm. The associating ratio was calculated as follows:

$$\text{Associating ratio} = (X_t - X_f)/X_t \times 100\%$$

where X_f is the free insulin in the supernatant, and X_t represents the total quantity of insulin added in the insulin nanoparticle colloid solution, which was also determined by the high performance liquid chromatography method.

2.5. In vitro release of insulin from the nanoparticles

In vitro release of insulin from the polybutylcyanoacrylate nanoparticles was studied using a dialysis system comprising a dialysis bag and receptor chamber. Briefly, 5 ml of insulin nanoparticles colloid solution was put into a dialysis bag, and 5 ml of 0.9% NaCl solution was added. Then, the dialysis bag was put into a 250 ml flask containing 100 ml of 0.9% NaCl solution (pH 7). The whole apparatus was placed in a water bath shaker thermostated at 37°C, and shaken at 50 cpm. The concentration of insulin in the receptor chamber was periodically determined by the above-mentioned method.

2.6. Determination of blood glucose level

Blood samples after coagulation were centrifuged at 3000 r·min^{-1} for 10 min at 4°C, and the blood glucose level was determined immediately with 20 μl serum sample according to glucose oxidase method (Okumura et al., 1992).

2.7. Intratracheal delivery of insulin nanoparticles

Male Sprague–Dawley rats, weighing 250–350 g fasted overnight were anesthetized with sodium pentobarbital (40 mg·kg^{-1}, i.p.) during the experiments. After the animal had been secured on its back on an animal surgery board, the trachea was exposed and then an incision was made between the fifth and sixth trachea rings caudal to the thyroid cartilage. For intratracheal delivery of drugs, a microsyringe was inserted through the incision to a depth of 12–15 mm according to the method of Enna and Schanker (1972). Insulin nanoparticle colloid solution, insulin solution or the control solution (PBS, pH 7) was injected directly into the trachea. The rats were maintained at an angle of 90° to horizontal for 30 s after the administration, then at 15° during the subsequent experiments.

To determine the relative pharmacological bioavailability in each experiment, insulin solution (5 IU·kg^{-1}, pH 7) was subcutaneously administered by bolus injection. Experiments were performed using five or six rats in each group in order to afford meaningful comparisons.

2.8. Data analysis

The pulmonary delivery of insulin nanoparticles was evaluated by its hypoglycemic effect. The area above the blood glucose curve (AAC) was calculated by the trapezoidal method. The relative pharmacological availability (PA%) was obtained by comparing the AAC following pulmonary administration with that following subcutaneous administration.

3. Results

The mean size of insulin-loaded nanoparticles was 254.7 nm, with a polydispersity of 0.064. About 4.5% of the insulin-loaded nanoparticles distributed between 160 and 250 nm, and 90.9% of them between 251 and 333 nm, while another 4.5% distributed between 334 and 400 nm. The size of insulin-loaded nanoparticles showed a normal distribution profile and the symmetry of the peak indicated a uniform distribution. The associating ratio of insulin in nanoparticles was 79.1%.

The release profiles of insulin-loaded nanoparticles at pH 7.0 and insulin solution at pH 7.0 in saline at 37°C were given in Fig. 1. The results showed that in vitro release of insulin from nanoparticles is characteristically biphasic, with an initial fast release (the burst effect), followed by a much slower release. These release profiles can be

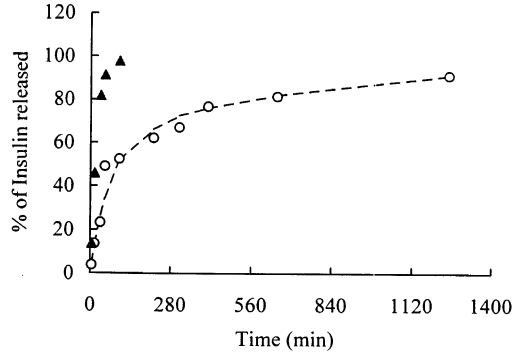

Fig. 1. Release profiles of insulin-loaded nanoparticles at pH 7 (○) and insulin solution at pH 7 (▲) in saline at 37°C. Scatter: observed; line: calculated.

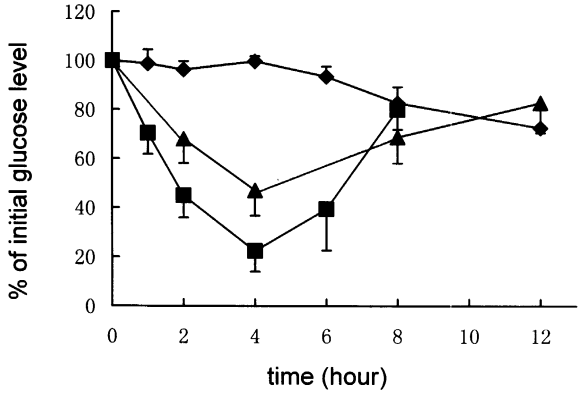

Fig. 2. Hypoglycemic effect of a single intratracheal administration of insulin-loaded nanoparticles of 5 IU·kg^{-1} (▲) and insulin solution of 5 IU·kg^{-1} (■) to normal rats fasted overnight. The glucose concentration at time zero served as the basis for comparison (%). Results are means for 5 or 6 animals. The control (◆) is phosphate buffer solution (pH 7).

well modeled using a biexponential function. The related parameters of release kinetics have been calculated according to this function, thence the equation of release kinetics of insulin-loaded nanoparticles was obtained as follows:

$$Y = 1 - [50.69\exp(-0.022t) + 49.85\exp(-0.002t)]$$

where Y is the % of insulin released, and t represents the time.

The hypoglycemic effects after pulmonary delivery of insulin-loaded nanoparticles were summarized in Figs. 2–4. The effectiveness between different doses of insulin-loaded nanoparticles and the insulin solution were compared. After pulmonary administration of 5 IU·kg^{-1} insulin-loaded nanoparticles or the insulin solution to normal rats, as shown in Fig. 2, the blood glucose concentration dropped to the minimum point of 46.9% and 22.3%, respectively. The time to reach the minimum blood glucose level (T_{min}) for both formulations was 4 h, but the hypoglycemic effect kept longer in the former situation than the latter. As seen in Fig. 3, when 10 IU·kg^{-1} insulin-loaded nanoparticles or the insulin solution was administered by pulmonary delivery to normal rats, the blood glucose level decreased to the minimum point of 30.4% with a T_{min} of 4 h and 20.2% with

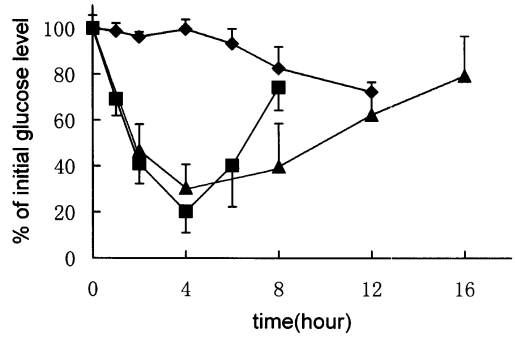

Fig. 3. Hypoglycemic effect of a single intratracheal administration of insulin-loaded nanoparticles of 10 IU·kg^{-1} (▲) and insulin solution of 10 IU·kg^{-1} (■) to normal rats fasted overnight. The glucose concentration at time zero served as the basis for comparison (%). Results are means for 5 or 6 animals. The control (◆) is phosphate buffer solution (pH 7).

a T_{min} of 2 h, respectively. The hypoglycemic effect of insulin-loaded nanoparticles lasted much longer than the insulin solution. When 20 IU·kg^{-1} of insulin-loaded nanoparticles or the insulin solution was administered as given in Fig. 4, the blood glucose level reached a minimum point of 13.6% at 8 h and 4.3% at 6 h, respectively. The difference of the two treatments in the action duration was also very significant. The hypoglycemic effect of insulin-loaded nanoparticles and insulin solution increased significantly as the dose of insulin increased.

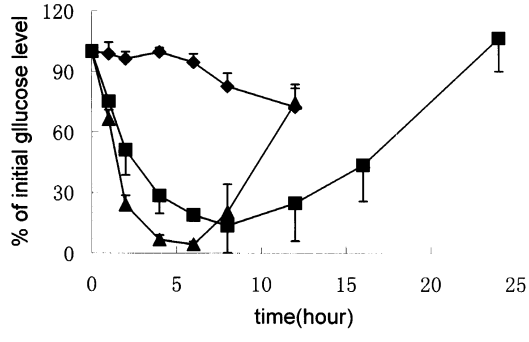

Fig. 4. Hypoglycemic effect of a single intratracheal administration of insulin-loaded nanoparticles of 20 IU·kg^{-1} (■) and insulin solution of 20 IU·kg^{-1} (▲) to normal rats fasted overnight. The glucose concentration at time zero served as the basis for comparison (%). Results are means for 5 or 6 animals. The control (◆) is phosphate buffer solution (pH 7).

Table 1
Duration of glucose level below 80% after pulmonary delivery of different doses of insulin-loaded nanoparticles and insulin solution to normal rats

Dose ($IU \cdot kg^{-1}$)	INS-SOL[a] (h)	INS-NP[a] (h)	Difference (h)
5	7.4	10.8	3.4
10	7.6	15.0	7.4
20	11.9	20.0	8.1

[a] INS-SOL, insulin solution; INS-NP, insulin-loaded polybutylcyanoacrylate nanoparticles.

The duration of glucose levels below 80% was estimated as a criterion to evaluate two insulin formulations, and such duration values after pulmonary delivery of different doses of insulin-loaded nanoparticles and insulin solution were summarized in Table 1. As listed in Table 1, the duration of glucose levels below 80% increased significantly as the dose of insulin increased for both insulin-loaded nanoparticles and insulin solution. But the values of insulin-loaded nanoparticles were obviously longer than that of insulin solution at every dose, and their difference increased as the dose of insulin increased. Prolonged hypoglycemic effects of insulin-loaded nanoparticles demonstrated the sustained release of insulin from the polybutylcyanoacrylate nanoparticles.

The comparison of hypoglycemic effects of insulin-loaded nanoparticles after pulmonary administration and subcutaneous administration was given in Table 2. The results suggested that bioavailability of insulin-loaded nanoparticles by pulmonary administration are lower than that by subcutaneous administration, and relative pharmacological bioavailability of insulin-loaded nanoparticles by pulmonary administration was 57.2% over that by subcutaneous administration.

4. Discussion

The associating ratio of insulin to nanoparticles was about 80%, suggesting that about 20% of free insulin exist in the insulin nanoparticle colloid solution. But in the in vitro release study, an initial fast release of about 50% of insulin released within the first 1.5 h from nanoparticles. It means not only the free part of insulin released at the initial phase, but also some of insulin absorbed on the surface of the polybutylcyanoacrylate nanoparticles released quickly from the surface of the nanoparticles, specially in the sink situation of the in vitro release.

Insulin loaded polybutylcyanoacrylate nanoparticles were prepared to investigate prolonged hypoglycemic effect of insulin after pulmonary delivery to normal rats. As demonstrated in our experiments, the pulmonary administration of this formulation to rats could significantly prolong the duration of hypoglycemic effect of insulin. This phenomenon could be explained by the combination of insulin molecule to the polybutylcyanoacrylate nanoparticles. The insulin molecule could be absorbed to, or embedded in, the solid polymer nanoparticles during the preparation procedure, so the release of insulin from the nanoparticle could be partly controlled. On the other hand, the particles in lung tissue could be taken up by the microphages (Geiser et al., 2000), may also resulted in a prolonged pharmacological action.

The duration of glucose levels below 80% was used as a criterion to evaluate insulin-loaded nanoparticles and insulin solution, because gener-

Table 2
Comparison of hypoglycemic effect of insulin nanoparticles after pulmonary administration and subcutaneous administration to normal rats ($n = 5$–6)

Time (h)	Control[a]	INS-NP[a] (5 $IU \cdot kg^{-1}$)	
		Intracheal	Subcutaneous
0	100.0	100	100
2	96.2 ± 3.5	67.9 ± 9.9	45.1 ± 4.8
4	99.6 ± 2.1	46.9 ± 10.3	26.2 ± 2.3
6	93.3 ± 4.2	51.2 ± 13.6	23.5 ± 2.6
8	82.6 ± 6.5	68.6 ± 10.7	24.8 ± 5.1
12	72.3 ± 9.3	82.6 ± 12.2	74.6 ± 4.1
AUC (h %)		406.9 ± 213.4	711.0 ± 106.2
PA[a] (%)		57.2	100

[a] Control, phosphate buffer solution (pH 7.0); INS-NP, insulin-loaded polybutylcyanoacrylate nanoparticles; PA, pharmacological availability.

ally speaking, hypoglycemic effect could be confirmed if the blood glucose level drop to or beyond 20% of the initial concentration.

As listed in Table 2, the bioavailability of insulin-loaded nanoparticles by pulmonary administration of 5 IU·kg^{-1} dose are lower than that by subcutaneous administration, and the relative pharmacological bioavailability of insulin-loaded nanoparticles by pulmonary administration reached 57.2% over the one by subcutaneous administration. In our previous study (Shen et al., 2000), the relative pharmacological bioavailability of insulin solution (pH 7) by pulmonary administration came to only 26.01% over the same insulin solution by subcutaneous administration. There may exist two possibilities. First, the relative bioavailability of insulin-loaded nanoparticles is higher than that of insulin solution after pulmonary administration to normal rats, and secondly, the relative bioavailability of insulin-loaded nanoparticles is lower than that of insulin solution after subcutaneous administration. But as shown in Fig. 2, the area above the blood glucose curve of insulin-loaded nanoparticles is not larger than that of insulin solution after pulmonary administration, indicating the bigger possibility of the latter situation.

As demonstrated in Fig. 2, when a low dose was administered to normal rats, the area above the blood glucose curve of insulin-loaded nanoparticles is slightly less than that of insulin solution. However, as the dose of insulin increased to 10 or 20 IU·kg^{-1}, the area above the blood glucose curve of insulin-loaded nanoparticles is obviously larger than that of insulin solution, as demonstrated in Figs. 3 and 4. It suggests that the relative bioavailability of insulin-loaded nanoparticles is larger than insulin solution at higher doses.

As seen in Figs. 2 and 3, the blood glucose profiles after pulmonary administration of 5 and 10 IU kg^{-1} insulin solution were almost the same, whereas the profile after administration of 20 IU kg^{-1} showed a great pharmcolocical action. The possible reasons for this phenomenon might include the physiological diversity in animals, variances in experiments and the ability of normal rats in adjusting its blood glucose level.

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