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Oral and subcutaneous absorption of insulin poly(isobutylcyanoacrylate) nanoparticles

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Abstract

Dispersions of insulin poly(isobutylcyanoacrylate) nanoparticles were obtained by anionic in situ polymerization using aqueous pluronic acid solution. Results showed a decrease in particle size diameter by increasing the pluronic acid concentration. Nanoparticles prepared in the presence of 2.5% pluronic acid resulted in particles of 85 nm average diameter and 59% intraparticular insulin load without the use of the oily core [Damge, C., Michel, M., Aprahamian, M., Couveur, P., 1988. New approach for oral administration with polycyanoacrylate nanocapsules as drug carrier. Diabetes 37, 246–251]. In vivo testing was performed on streptozocin induced diabetic rats. The subcutaneous injection of insulin nanoparticles was able to prolong its duration of hypoglycemic effect from 6 to 72 h. Effective oral absorption of the entrapped insulin was significantly better ($p < 0.01$) when compared with non-encapsulated insulin or the control experiments.

Keywords: Insulin; Oral insulin; Insulin nanoparticles; Polyisobutylcyanoacrylate; Streptozocin-induced diabetes; Prolonged release parenteral insulin; Pluronic acid

1. Introduction

The use of nanoparticles as a drug delivery system was found to be useful in prolonging the duration of insulin effect. Limited enhancement of oral insulin absorption in polymeric nanocapsules was reported (Aboubakar et al., 1999; Couvreur et al., 1979, 1980; Speiser, 1974). Polymerization was reported to be associated with problems such as radiation and the poor biodegradability of acrylic nanoparticles prepared (Couvreur et al., 1979, 1980; Kreuter, 1978, 1983). Radiation was performed to break chemical bonds in the material to be polymerized, grafted, forming free radicals, or other reactive species. Ionizing radiation source most commonly a cobalt-60 gamma radiation source (Hoffman et al., 1983; Ratner, 1980). Lanerts et al. (1984) found a correlation between the rate of degradation and the chain length of the polymer.

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Oil dispersion in the emulsion polymerization of nanocapsules was effective to enhance oral absorption of insulin specially by reducing size down to 145 nm (Damge et al., 1988). Other restrictive parameters affecting the ability of insulin to be loaded in oil containing nanocapsules are the pH of aqueous solution and the origin of the monomer (Cournarie et al., 2004).

The objective of this study was to include insulin during the stage of polymerization at very beginning, increasing the concentration while achieving the polymerization using pluronic acid, trying to produce submicroscopic polymeric particles to enclose the insulin. Both the oral and subcutaneous injection of the nano-dispersion of insulin poly(isobutylcyanoacrylate) (PIBCA) was studied using the diabetic rats as test animals.

2. Materials and methods

2.1. Materials

Regular human insulin (Humulin®-R, U-100, Lilly), isobutylcyanoacrylate, pluronic acid F-68, buffer components, streptozocin and other chemicals were analytically pure grades from Sigma Co. (St. Louis, MI). Acetonitrile, phosphoric acid, sodium monophosphate, water and other chemicals used in the assay of insulin were HPLC analytical grades from Sigma Co. (St. Louis, MI).

2.2. Animals and diabetes induction

Six weeks old, male Wistar rats were used, weighing 150–200 g. They were kept to meet IACUC approved protocol, with normal day/night time variation, and access to water ad libitum. Diabetes was induced in the rats by a single intravenous injection of streptozocin 65 mg/kg; in solution prepared in normal saline in the tail vein. After 8–10 days, the rats showed symptoms of frequent urination and loss of weight. They were considered diabetic when fasting blood glucose level was 250 mg/dL or higher. Blood samples were collected from the animals' tail vein and analyzed for glucose content using Glucometer® II (Ames) with the standardized test strips supplied with the apparatus.

2.3. Preparations of insulin nanoparticles

Nanoparticles were prepared by a modified polymerization method of Couvreur et al. (1990) as the oil phase was not applied. Two milliliters of insulin solution of starting pH 7.42 ± 0.02, equivalent to 200 U were added to a solution containing different concentrations of pluronic acid, ranging from 0.5 to 2.5%, at lowest pH of 1.7, and 2 mL of 5% (w/v) citric acid solution. Accurately measured 500 μL of isobutylcyanoacrylate was added drop wise while mixing to every 10 mL of insulin solution. The mixture was then stirred for 4 h at room temperature using a magnetic stirrer. The resulting milky dispersion was neutralized with 1.0 N sodium hydroxide solution to pH 7.4 ± 0.10.

2.4. Properties of insulin-nanopaticles

The particle size and size distribution of the particles were monitored using a Nicomp submicron laser diffraction size analyzer. The size of the obtained nanoparticles was correlated with the concentration of the pluronic acid used.

Insulin loading inside the particles was monitored by analyzing the extra particulate insulin concentration, using an HPLC method (Sidhom et al., 1995). The distribution was confirmed by electron microscopy.

2.5. In vivo evaluation of insulin-nanoparticles

Kinetics of insulin absorption was monitored by its hypoglycemic effect in the streptozocin induced diabetic rats, obtained as explained above. Animals were fasted overnight with water ad libitum. They were fed during the experiments using a controlled schedule of commercial chow (Purina, 5008; 6.5% fat) twice daily. Subcutaneous experiments were performed on three groups of animals: control nanoparticles with no insulin, insulin loaded nanoparticles, and regular insulin. The nanoparticles were injected to the rats subcutaneously in the dispersion medium including the free unloaded insulin, at a dose of 5 U/kg, while the regular insulin was injected at a dose of 2.5 U/kg body weight. Thus, the dose of free insulin from nanoparticles formulation was about the same as the dose administered from the comparative regular insulin injection. Oral insulin dispersions were given to fasting diabetic rats through

a stomach tube at a dose equivalent to 100 U free insulin/kg body weight. Control animals were treated similarly except for placebo polymer that was prepared with no insulin. First scheduled meal was offered after the induction of test preparation.

2.6. Statistical analysis

The animals were used in-groups of five using a Latin square crossover design. One-way analyses of variance (ANOVAs) were followed by multiple comparison with Student Newman–Keul's tests. Blood glucose measurements were recalculated as percent relative to the initial blood sugar levels; and are expressed as mean ± S.D. used for the statistical analysis of potential differences from control results. $p < 0.05$ is regarded as significant.

3. Results and discussion

The problem of encapsulating insulin in the alkylcyanoacrylate polymers was mainly due to its large molecular mass. Previously published technique (Couvreur et al., 1980; Damge et al., 1988) of nanoparticle preparation resulted in limited drug loading 47% of the dose became bioavailable only after subcutaneous use over 36 h or more (Damge et al., 1988). The molecules of insulin encapsulated were slowly diffusing to the surface of the particles (Couvreur et al., 1980). The possibility of better bioavailability of insulin from the nanoparticles is thought to be a function of particle size (Damge et al., 1988). It was the main objective of this work to prepare nanoparticles of insulin in PIBCA of less than 100 nm diameter size, without using the oily core to entrap the insulin, characterize the prepared form of insulin and study the hypoglycemic effect of orally and subcutaneously administered drug on laboratory animals.

Chromatographic analysis of insulin encapsulated, expressed as the percent load, resulted in encapsulated insulin ranging from 53 to 59% in the nanoparticles (Table 1). The particles were homogeneous and round in shape under the microscope, figure is not shown. The nanoparticles with 2.5% pluronic acid used in polymerization were those selected for in vivo bioavailability studies, especially because of our target particle size was below 100 nm in average diameter.

The surface activity of pluronic acid at higher concentration resulted in particle size smaller than 100 nm in diameter, of oleaginous core when small enough it could replace the oily core in emulsion polymerization (Damge et al., 1988; Cournarie et al., 2004).

Subcutaneous injection of the insulin was tested in vivo on diabetic rats of average initial fasting blood glucose level of 296 mg/dL. Non-insulin control experiments resulted in slight increase of the initial blood glucose level from 100% (268–344 mg/dL; $n = 5$) up to 108–129% ± 0.91 during the experiment time. The injection of regular insulin (2.5 U/kg) resulted as seen in Fig. 1 in short duration of hypoglycemic effect that started gradually within 1–2 h, reaching maximum in 6 h time, then back to normally hyperglycemia within 8 h; as the animal were kept on food during experiment. They were fed during the experiments using a controlled schedule to minimize variations and prevent hypoglycemic reactions. The insulin nanoparticles were injected at higher dose since almost 60% of the insulin was found to be loaded. The subcutaneous administration resulted in initial sharp decline in blood sugar level likely due to the free unbound insulin. The hypoglycemic effect was extended over 6–72 h, a gradual hypoglycemic combined effect of the free and the encapsulated insulin, continued by the extended release of insulin from the polymeric core, when the blood sugar level returned to its initial high level. The results indicate possible normoglycemic level with lower subcutaneous dose of the nanoparticles, was not

Table 1
Particle size of nanoparticles as a function of pluronic acid concentration, its possible effects on insulin loading ($n = 5$)

Pluronic acid (%, w/v)	Average size (nm)	Coefficient of variation	Percentage load encapsulated	Coefficient of variation
0.5	182.2	±0.193	54	±0.202
1.0	156.0	±0.088	56	±0.710
1.5	136.3	±0.274	54	±0.184
2.0	97.0	±0.310	53	±0.440
2.5	85.2	±0.315	59	±0.245

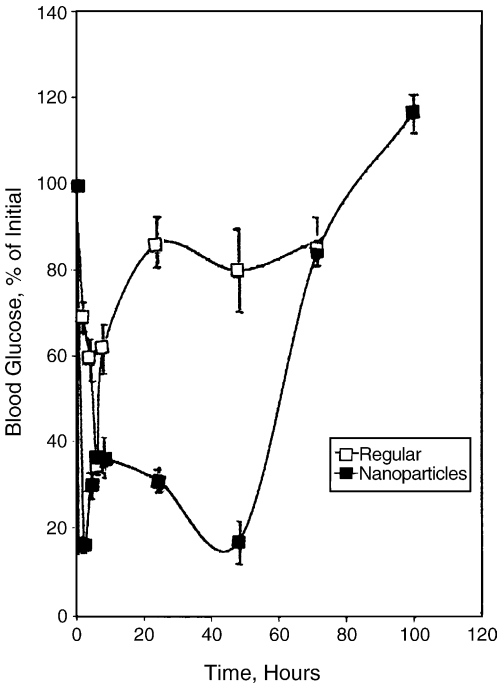

Fig. 1. Hypoglycemic effect of the Regular insulin (2.5 U/kg) and insulin nanoparticles (5 U/kg) injected subcutaneously to diabetic rats (Fasting blood glucose level: 220–409 mg/dL). Results are means ± S.D. of five animals per group.

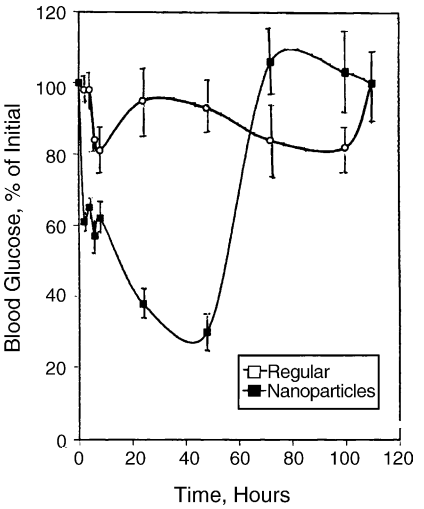

Fig. 2. Hypoglycemic effect of the regular insulin and insulin nanoparticles given orally to diabetic rats (fasting blood glucose level: 207–368). Results are means ± S.D. of five animals per group.

tried due to limits of the number of animals we could use.

Results of oral nanoparticles with no insulin showed initial blood glucose level, considered as 100% to range between 250 and 409 mg/dL. The values fluctuated during the experiment between 102 and 119% ± 1.82 ($n = 5$). Oral insulin nanoparticles demonstrated fast start with extended effect as shown in Fig. 2. Hypoglycemic effect started two hours after its administration at a significantly lower blood glucose level. Less prominent effect was given by the same dose of insulin in nanocapsules with oily core (Damge et al., 1988). The fast absorption may be attributed to better chance of the ultra fine particle size, below 100 nm to be entrapped between the villi and microvilli of intestine. Dimension of particles close to the natural chyles are expected to be easier in absorption (Mesiha et al., 2002).

Oral doses (100 U/kg) used to achieve the shown effect, included about 40% extra-particulate insulin which was not bioavailable, was not separated from the media and would be liable for enzymatic degradation or other form of protein deformation in the gut. The relative efficacy of the nanoparticles through oral route when compared with unloaded insulin or the control experiments for lowering the blood glucose level indicated significant difference in second hour of experimental data extended over 40 h ($p < 0.01$–$p < 0.02$). The area above the glucose concentration–time plot and below the baseline assuming the initial blood glucose level of the control group as baseline, when cross examined it gives significant difference, $p < 0.01$.

The in vivo difference between the two dosage forms was mainly due to the bioavailability of extra-particulate insulin when injected subcutaneously. This unloaded insulin – about 40 U/kg of the experimental dose – did not have any mechanism of absorption enhancement or enzymatic protection. In the absorption studies we monitored the absorption of biologically active intact insulin through its hypoglycemic effect.

In conclusion insulin entrapped in in situ poly-isobutylcynoacrylate nanometer dimension particles using high pluronic acid concentrations without the need of oily core. The nanoparticles were tested on streptozocin induced diabetic rats. The subcutaneous injection of insulin nanoparticles was able to prolong its duration of hypoglycemic effect from 6 to 72 h for the subcutaneous insulin nanoparticles. Oral absorption

of the entrapped insulin was significantly enhanced ($p < 0.01$) when compared with non-encapsulated insulin or the control experiments.

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