Design Improvements to Insulin In Vitro Release and Oral Bioavailability via Nanoparticle Mediated Delivery System

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INTRODUCTION AND OBJECTIVES

Since the discovery of insulin, parenteral dosage form is the only form available on the market. In spite of its prevalence, there are several concerns associated with this mode of administration including injection anxiety, pain, cost, infection, and an overall decrease in patient compliance compared to other, more widely accepted methods of delivery such as oral or transdermal (Khafagy 2007). This has encouraged researchers to search for alternative delivery methods. Among the delivery routes, administration via the oral route is preferred because it is simple, non-invasive and convenient (Sintov 2007). Oral administration however is difficult due to low bioavailability. One such development is the use of polymeric biodegradable and biocompatible nanoparticles, which protect insulin from degradation and facilitate uptake through a paracellular or a transcellular pathway. Our overall objective is to improve the efficacy involved with nanoparticle-based oral delivery systems for insulin.

MATERIALS AND METHODS

Preparation of Insulin Nanoparticles

Method 1: Ionotropic gelation/polyelectrolyte complexation (IG/PC)

A starting solution was prepared containing 0.063% (w/v) alginate, 0.039% (w/v) dextran sulfate, 0.037% (w/v) poloxamer 188, and 7 mg insulin. The solution was adjusted to a pH of 4.9 before 7.5 mL of 18 mM calcium chloride was added dropwise over the course of 60 min. The nanoparticle cores were coated in 0.04% (w/v) chitosan solution. Finally, the nanoparticles were coated with bovine serum albumin (BSA) at a pH of 5.1. (Woitiski 2009)

Method 2: Nanoemulsion dispersion (ND)

Insulin (100 IU/mL, 10 mL) was added to a mixture of sodium alginate (2%, w/v containing ultrafine calcium carbonate; 5%, w/v) and dextran sulfate solution (0.75%, w/v). The mixture was emulsified within paraffin oil facilitated by Span 80 emulsifier (2.5% v/v) at high speed. After 15 min emulsification, gelation was induced by addition of 20 mL paraffin oil containing glacial acetic acid (acid/ca molar 3 :1) to solubilize calcium dispersed in the alginate dextran nano droplets. After separating the particles from the emulsion, nanoparticle cores were coated in PEG-chitosan solution. Finally the nanoparticles were coated with bovine serum albumin (BSA) at a pH of 5.1.(Reis 2008)

Characterization of Insulin Nanoparticles

Characterization included SEM analysis and particle size measurement.

Encapsulation efficiency was measured by separating insulin nanoparticles from aqueous medium by centrifugation at 20,000g for 60 min at 4C. The amount of unassociated insulin in the supernatant was determined by HPLC (Woitiski 2009).

Insulin hypoglycemic effect and bioavailability

Hypoglycemic effect and insulin nanoparticle bioavailability were evaluated by the decrease of plasma glucose levels and the serum insulin level in comparison with the basal values in 12 h fasted diabetic rats, gavaged with insulin nanoparticles at different doses or empty nanoparticles as control. Blood samples were taken from the tail vein during 24 h. The serum insulin measurement was determined by using Iso-insulin ELISA. The blood serum was separated by centrifugation at 2000 rpm for 15 min.

RESULTS AND DISCUSSION

Insulin-loaded nanoparticles formed with the IGPC method showed a uniform size distribution with mean diameter of 460 nm; and particles observed under the SEM were relatively smooth and spherical. Nanoparticles prepared by the ND method resulted in an unimodel distribution, with approximately 90% of the particles having a diameter less than 1842 nm, and 50% less than 812 nm as shown in the figure 1. An entrapment efficacy of 85% with the IGPC method was influenced by calcium and albumin concentration. The pharmacological effect of orally delivered nanoencapsulated insulin (50 IU/kg) in diabetic rats reduced the plasma level to 40% of the basal value and pharmacological availability of 13%, 12 h after oral administration as shown in table 1.

The EE of the ND formulation was evaluated as 90±4%. Glycemic response was examined by orally dosing rats with 25, 50, and 100 IU/kg.

Insulin nanoparticles decreased glycaemia in a dose-dependent manner in contrast to rats treated with empty nanoparticles as is illustrated in figure 2.

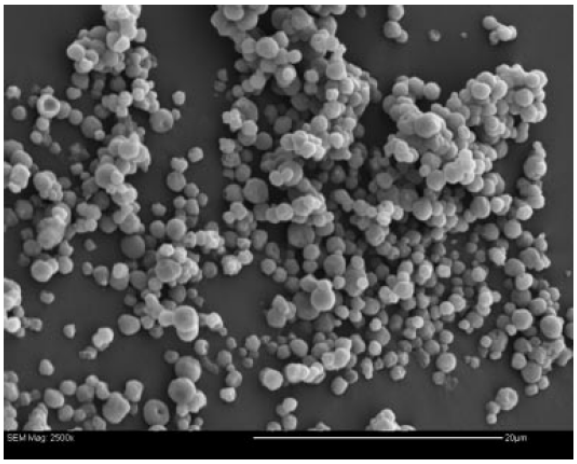

Figure 1.SEM of particles formed by ND method

The glycemic reductions were 44, 55, 76% with the 25, 50 and 100 IU/kg respectively. Pharmacological availability was calculated based on $AUC_{0\text{-}8h}$ (table 1) showing 42% for 25 IU/kg dose.

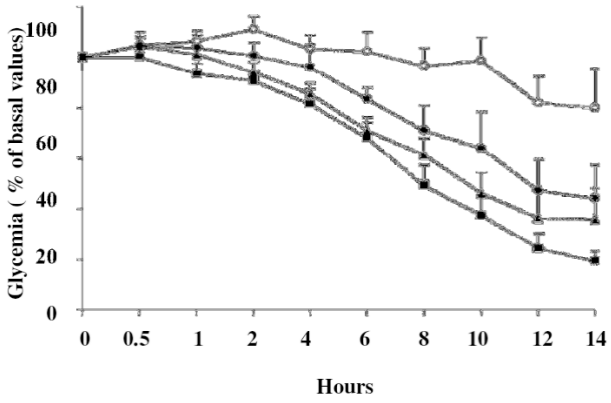

Figure 2. Glycemia after a single oral administration of ND nanospheres: 25 IU/kg (full circles, n=8), 50 IU/kg (triangles, n=9), and 100 IU/kg (squares, n=8) or empty nanospheres as control (empty circles, n=9) in fasted diabetic rats.

The role of gastric pH, gut proteases and premature insulin release were examined in a gastrointestinal tract simulation using the IGPC particles. All factors contributed to loss of insulin and insulin stability, likely contributing to considerably lower PA, compared to particles formed through the ND method. The next phase of the research will be to examine the ND formed nanoparticles to determine the factors responsible for reduced PA, so as to increase levels above the presently reported 42%.

CONCLUSIONS

In the oral delivery of the insulin nanoparticles, alginate-dextran nanoparticles coated with chitosan/PEG-albumin can play a promising role in preserving the biological activity of a protein drug, by protecting it during formulation and from proteolytic degradation during gastrointestinal transit.

Table1. Pharmacological availability of insulin nanoparticles administered to diabetic rats

Route	Dose(IU/kg)	$AUC_{0\text{-}8h}$	PA(%)
ND Method			
SC	4	265±18	100
Oral	25	696±43	42
Oral	50	670±31	21
Oral	100	630±36	10
IG/PC Method			
SC	5	160±34.8	100
Oral	50	121.4±18.7	13.2±2.9

Insulin nanoparticles with small size (50% less than 812 nm) and encapsulation efficiency of 90% were produced by emulsion dispersion/triggered gelation, followed by polyelectrolyte coating. Blood glucose reduction following oral administration was higher than 70% of the basal value in producing oral hypoglycaemic response. The formulation showed bioavailability around 42% for the dose 25 IU/kg, thus this formulation is able to dramatically improve the intestinal absorption of insulin and will be of interest in the treatment of diabetes with oral insulin.

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