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Research paper

Nasal absorption enhancement of insulin using PEG-grafted chitosan nanoparticles

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Abstract

The objective of this work was to explore the potential of polyethylene glycol-grafted chitosan (PEG-*g*-chitosan) nanoparticles as a system for improving the systemic absorption of insulin following nasal administration. Insulin-loaded PEG-*g*-chitosan nanoparticles were prepared by the ionotropic gelation of PEG-*g*-chitosan solution using tripolyphosphate ions as the crosslinking agent. The nanoparticles were in the size range 150–300 nm, had a positive electrical charge (+16 to +30 mV) and were associated with insulin (loading efficiency 20–39%). The physicochemical properties of nanoparticles were affected by the composition of the copolymer. *In vitro* insulin release studies showed an initial burst followed by a slow release of insulin. Intranasal administration of PEG-*g*-chitosan nanoparticles in rabbits enhanced the absorption of insulin by the nasal mucosa to a greater extent than a suspension of insulin-PEG-*g*-chitosan and control insulin solution. PEG-*g*-chitosan nanoparticles are promising vehicles for insulin transport through the nasal mucosa.

Keywords: PEG-*g*-chitosan; Nanoparticles; Nasal delivery; Insulin; Blood glucose; Absorption enhancement

1. Introduction

Oral delivery offers a comfortable and physiologically acceptable way to administer a wide range of drugs. However, macromolecular drugs such as peptides and proteins cannot be given orally because they are degraded by the proteolytic enzymes in the stomach, and most of these drugs need to be administered repeatedly by injection. Significant efforts have been made to explore alternative routes for drug administration. Intranasal drug delivery is a convenient and reliable method that has many advantages, such as a large absorptive surface area and high vascularity of the nasal mucosa, where drugs absorbed from the nasal cavity pass directly into the systemic circulation, thereby avoiding first-pass liver metabolism [1]. However, the administration of macromolecules by this route is hampered by the chemical and physical instability of these molecules, and by the high metabolic activity and limited permeability of the mucosal barriers [2].

There are many approaches to improving the absorption of peptides and proteins through the nasal mucosa by the use of absorption enhancers, enzyme inhibitors and solutions of bioadhesive polymers or bioadhesive microspheres [3–5]. The use of most absorption enhancers, such as surfactants, bile salts and fatty acids, is accompanied by mucosal damage [6]. However, the absorption-enhancing effect of the polysaccharide chitosan outweighs the damage caused to the nasal mucosa [7]. The mechanism of action of chitosan is suggested to be a combination of bioadhesion and a transient widening of the tight junctions between epithelial cells [8]. It should be noted that chitosan adsorbs plasma proteins in contact with blood, which leads to

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surface-induced thrombosis occurring at the blood-biomaterial interface [9].

PEG-*g*-chitosan copolymers have been synthesized in an attempt to increase the solubility and improve the biocompatibility of chitosan [10]. Moreover, modification of chitosan with PEG can resist adsorption of plasma proteins in contact with blood through the steric repulsion mechanism [9]. Insulin-PEG-*g*-chitosan nanocomplexes are formed by intermolecular hydrogen bonding in an aqueous solution [11]. On the basis of these results, we decided to explore the potential of PEG-*g*-chitosan nanoparticles as a delivery vehicle for nasal administration of proteins and peptides.

The goal of the present work was to associate insulin, as a model peptide, with PEG-*g*-chitosan nanoparticles. We first studied the preparation of insulin-loaded nanoparticles by using different formulation conditions and characterized their physicochemical properties and the *in vitro* release of insulin. Then, the ability to enhance the nasal absorption of insulin was investigated by determining the decrease in plasma glucose levels following nasal administration. Finally, we studied the effect of the composition of PEG-*g*-chitosan on the ability of the nanoparticles to transport insulin across the nasal mucosa in rabbits.

2. Materials and methods

2.1. Materials

Chitosan (degree of deacetylation 90%; with molecular mass of 6 and 20 kDa) was obtained from the Zhejiang Yuhuan Ocean Biochemical Co., Ltd. (Zhejiang, China). PEG350 (350 Da) and PEG750 (750 Da) were purchased from the Aldrich Chemical Co., Inc. (USA). Pure crystalline porcine insulin (with a nominal activity of 28 IU/mg) that was used without further purification was obtained from the Xuzhou Wanbang Biochemical Co., Ltd. (Jiangsu, China). All other chemicals were of analytical grade.

2.2. Synthesis of PEG-g-chitosan

PEG-*g*-chitosan was prepared as described by Harris et al. [12]. First, PEG-aldehyde was prepared by oxidation of PEG with DMSO/acetic anhydride. Acetic anhydride was added to the mixture under nitrogen after MeO-PEG was dissolved in anhydrous DMSO/chloroform (9:1, v/v) with acetic anhydride/PEG molar ratio of 12, and the mixture was stirred for 9 h at room temperature under nitrogen. The reaction mixture was neutralized with 1 M NaOH and used directly for the next reaction.

PEG-*g*-chitosan was prepared by alkylation of chitosan followed by Schiff base formation. PEG-aldehyde with different molar ratios to the amino groups of chitosan was added to a mixture of acetic acid and methanol (2:1, v/v), and the mixture was stirred for 30 min at room temperature. A solution of sodium tetrahydroborate ($NaBH_4$) was added slowly to the reactant mixture at pH 6.5 with vigorous stirring at a constant molar ratio of 10:1 for $NaBH_4$/PEG-aldehyde. The resultant mixture was dialyzed (3500 Da cutoff) first against 0.05 M NaOH and then distilled water before the solution was freeze-dried. PEG-*g*-chitosan was finally obtained by removal of unreacted PEG with excess acetone.

2.3. Preparation of PEG-g-chitosan nanoparticles

The preparation of PEG-*g*-chitosan nanoparticles based on the ionic gelation of chitosan with tripolyphosphate anions (TPP) was adapted from the method described by Calvo et al. [13]. Experiments were done to identify the production zone for formation of nanoparticles. For this purpose, PEG-*g*-chitosan was dissolved in (2%, w/v) acetic acid at various concentrations of PEG-*g*-chitosan (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 7.0, and 9.0 mg/ml). TPP was dissolved in purified water at the same concentrations as PEG-*g*-chitosan. Finally, various volumes of TPP solution (0.5, 1.0, 2.0, and 3 ml) were added dropwise to 5 ml of PEG-*g*-chitosan solution with stirring at room temperature. The samples were analyzed visually and three different systems were identified: clear solution, opalescent suspension (nanoparticles) and aggregates. The opalescent suspension, which should correspond to a suspension of very small particles, was investigated further by adding 2 ml of TPP solution to 5 ml of PEG-*g*-chitosan solution, thus achieving a final concentration of PEG-*g*-chitosan between 0.38 and 2.86 mg/ml and a final concentration of TPP between 0.28 and 1.14 mg/ml. The appearance of these preparations was observed microscopically and samples were classified as aggregates or nanoparticles, as shown in Fig. 1. To evaluate the effect of PEG-*g*-chitosan on the nasal absorption of insulin, further experiments were conducted using a PEG-*g*-chitosan final concentration of

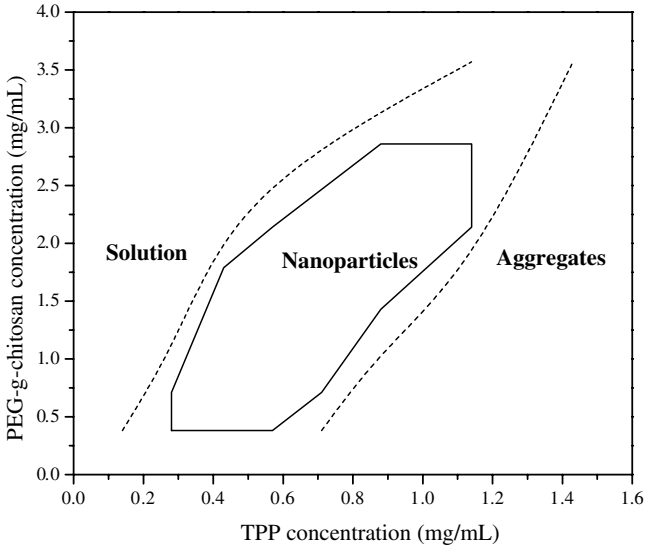

Fig. 1. Identification of PEG-*g*-chitosan and TPP concentrations for the formation of nanoparticles.