Enhancement of Nasal Absorption of Insulin Using Chitosan Nanoparticles

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Purpose. To investigate the potential of chitosan nanoparticles as a system for improving the systemic absorption of insulin following nasal instillation.

Methods. Insulin-loaded chitosan nanoparticles were prepared by ionotropic gelation of chitosan with tripolyphosphate anions. They were characterized for their size and zeta potential by photon correlation spectroscopy and laser Doppler anemometry, respectively. Insulin loading and release was determined by the microBCA protein assay. The ability of chitosan nanoparticles to enhance the nasal absorption of insulin was investigated in a conscious rabbit model by monitoring the plasma glucose levels.

Results. Chitosan nanoparticles had a size in the range of 300–400 nm, a positive surface charge and their insulin loading can be modulated reaching values up to 55% [insulin/nanoparticles (w/w): 55/100]. Insulin association was found to be highly mediated by an ionic interaction mechanism and its release in vitro occurred rapidly in sink conditions. Chitosan nanoparticles enhanced the nasal absorption of insulin to a greater extent than an aqueous solution of chitosan. The amount and molecular weight of chitosan did not have a significant effect on insulin response.

Conclusions. Chitosan nanoparticles are efficient vehicles for the transport of insulin through the nasal mucosa.

KEY WORDS: absorption enhancement; chitosan; insulin; nanoparticles; nasal delivery.

INTRODUCTION

Nowadays, several mucosal surfaces such as the nasal, pulmonary, and peroral mucosa are being extensively explored as alternative routes for the systemic administration of macromolecular drugs. Among them, the nasal mucosa is receiving a great deal of attention due to its particularly high permeability and the easy access of the drug to the absorption site (1). The efficacy of the nasal route for the absorption of large peptides, such as insulin is low (2,3). Several absorption enhancers, including surfactants, protease inhibitors, solutions of bioadhesive polymers or bioadhesive microspheres have been proposed to overcome this limitation (4–9). In general, drug absorption enhancement is accompanied by mucosal damage (10–12). An exceptional behavior has, however, been observed for the polysaccharide chitosan (13). The mechanism of action of chitosan was suggested to be a combination of bioadhesion and a transient widening of the tight junctions between epithelial cells (14).

We have recently developed a new type of chitosan nanoparticles obtained by a very mild ionotropic gelation procedure, and have reported their excellent capacity for the association of proteins, such as BSA, tetanus toxoid and dihydrotoxin (15–16) or oligonucleotides (17). Based on the acceptability of chitosan for nasal administration, it was our idea to explore the potential of chitosan nanoparticles as a delivery vehicle for nasal administration of proteins and peptides. The hypothesis behind this idea was that chitosan nanoparticles would intensify the contact between the protein and the nasal absorptive mucosa, thus leading to an increased protein concentration at the absorption site.

Therefore, our first aim was to associate efficiently the model peptide insulin to chitosan nanoparticles. We chose the model peptide insulin because of the simplicity of measuring its therapeutic response, i.e., blood glucose concentration. Insulin-containing nanoparticles were prepared using different formulation conditions and characterized for their physicochemical properties and in vitro release behavior. Secondly, their ability to enhance the nasal absorption of insulin was studied by determining the decrease in the plasma glucose levels following nasal instillation.

MATERIALS AND METHODS

Materials

Two types of chitosan, in the form of hydrochloride salt (Seacor® 210 CI and Protasan® 110 CI) were purchased from Pronova Biopolymer, A.S. (Norway). Their main physicochemical characteristics are summarized in Table I. Pentasodium tripolyphosphate (TPP) and bovine insulin were supplied by Sigma Chemical Co. (USA). Ultrapure water (MilliQ Plus, Millipore Iberica, Spain) was used throughout. All other chemicals were reagent grade.

Methods

Preparation of Chitosan Nanoparticles

Chitosan nanoparticles were prepared according to the procedure previously developed by our group, based on the ionotropic gelation of chitosan with TPP anions (15). Ionotropic gelation happens when the positively charged amino groups in chitosan interact with the negatively charged TPP. Chitosan 210 CI and 110 CI were dissolved in purified water at 0.10%, 0.15%, 0.20%, and 0.25% (w/w). TPP was also dissolved in purified water at various concentrations in order to obtain a final ratio chitosan/TPP of 8/1, 7/1, 6/1, 5/1 and 4/1 (w/w). The nanoparticles were formed spontaneously upon the incorporation of a variable volume of the TPP solution into 3 ml of the chitosan solution, under magnetic stirring at room temperature.

For the association of insulin to chitosan nanoparticles, insulin was dissolved in 0.01N NaOH (5 mg/ml) and then incorporated in the TPP solution. The concentration of insulin
Table 1. Physicochemical Characteristics of Chitosan (Hydrochloride Salts)

<table>
<thead>
<tr>
<th></th>
<th>Chitosan 110 Cl (Protasan® 110 Cl)</th>
<th>Chitosan 210 Cl (Seacure® 210 Cl)</th>
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</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>(X 1000)</td>
<td>&lt; 50</td>
</tr>
<tr>
<td>Degree of deacetylation (%)</td>
<td>87</td>
<td>108</td>
</tr>
<tr>
<td>Intrinsic viscosity</td>
<td>(mPas, 25°C)</td>
<td>10</td>
</tr>
<tr>
<td>pH of chitosan solution</td>
<td>4.81</td>
<td>4.63</td>
</tr>
</tbody>
</table>

...in the TPP solution was calculated in order to obtain nanoparticles with a 20%, 30%, 40%, and 50% (w/w) of insulin based on chitosan.

...in vitro characterization or in acetate buffer (pH 4.3) or phosphate buffer (pH 6.4) for the in vivo studies.

Characterization of Nanoparticles

The morphological examination of the nanoparticles was performed by transmission electron microscopy (TEM) (CM12 Philips, Eindhoven, Netherlands). The samples were stained with 2% (w/v) phosphotungstic acid and placed on copper grids with Formvar® films for viewing by TEM.

...a Zetasizer® III (Malvern Instruments, Malvern, UK). For the determination of the electrophoretic mobility, samples were diluted with KCl 0.1 mM and placed in the electrophoretic cell where a potential of ±150 mV was established. Each batch was analyzed in triplicate.

Insulin Loading Capacity of the Nanoparticles

The association efficiency of the process was determined upon separation of nanoparticles from the aqueous medium containing the nonassociated insulin by ultracentrifugation at 16,000 × g at room temperature, for 30 min. The amount of free insulin was measured in the supernatant by the microBCA protein assay (Pierce, Rockford, USA). A calibration curve was made using the supernatant of blank nanoparticles. Each sample was assayed in triplicate. The insulin loading capacity of the nanoparticles and the insulin association efficiency were calculated as indicated below.

Loading capacity

\[ \text{Loading capacity} = \frac{\text{Total amount of insulin} - \text{Free insulin}}{\text{Nanoparticles weight}} \times 100 \]

Association efficiency

\[ \text{Association efficiency} = \frac{\text{Total amount of insulin} - \text{Free insulin}}{\text{Total amount of insulin}} \times 100 \]

...the required dose of insulin (5 IU/Kg) was administered in a volume range of 130–170 μl to the rabbits, depending on the insulin loading of the nanoparticles and animal weight. The amount of nanoparticles instilled varied between 0.40–0.64 mg/kg.

...the mean blood glucose levels determined in samples collected before insulin administration were taken as the baseline levels. Using these values, the percentage of glucose reduction at each time after dosing was calculated and plotted against.