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Oral delivery of insulin via polyethylene imine-based nanoparticles for colonic release allows glycemic control in diabetic rats

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ABSTRACT

In this study, insulin-containing nanoparticles were loaded into pellet cores and orally administered to diabetic rats. Polyethylene imine-based nanoparticles, either placebo or loaded with insulin, were incorporated by extrusion and spheronization technology into cores that were subsequently coated with three overlapping layers and a gastroresistant film. The starting and coated systems were evaluated *in vitro* for their physico-technololgical characteristics, as well as disintegration and release performance. Nanoparticles-loaded cores showed homogeneous particle size distribution and shape. When a super-disintegrant and a soluble diluent were included in the composition enhanced disintegration and release performance were observed. The selected formulations, coated either with enteric or three-layer films, showed gastroresistant and release delayed behavior *in vitro*, respectively. The most promising formulations were finally tested for their hypoglycemic effect in diabetic rats. Only the nanoformulations loaded into the three-layer pellets were able to induce a significant hypoglycemic activity in diabetic rats. Our results suggest that this efficient activity could be attributed to a retarded release of insulin into the distal intestine, characterized by relatively low proteolytic activity and optimal absorption.

1. Introduction

Current therapy for diabetes mellitus relies on a correct diet, physical exercise, and oral hypoglycemic agents [1]. In case of disease progression or in Type 1 diabetes, insufficient insulin secretion or inadequate activity need to be considered. Therefore, replacement with exogenous insulin becomes mandatory for survival [1,2]. Unfortunately, nowadays the only administration route available for insulin is parenteral which implies one or more daily injections, with a significant reduction in quality of life and, sometimes, poor patient adherence to therapy [3,4]. Moreover, administration of insulin by subcutaneous injection may induce peripheral hyperinsulinaemia and portal hypoinsulinaemia. Under normal conditions, half of insulin produced by the pancreas is used for liver metabolism via the portal circulation, resulting in fine regulation of blood glucose levels and adequate metabolism of carbohydrates and proteins [2]. Thus, several research studies have been focused on the development of novel formulations of the hormone through alternative routes of administration [5]. Particularly, the oral route has been considered as possibly leading to a better glucose regulation exploiting the liver first-pass metabolism of insulin, thus preventing the risks of fluctuating blood glucose levels and possibly the resulting morbidity due to chronic microvascular complications [6]. Therefore, an oral formulation of insulin could revolutionize the management of insulin-dependent diabetic patients due to its potential clinical benefits. However, the oral bioavailability of insulin is very low and several efforts have been attempted to promote insulin bowel absorption, avoiding gastric or intestinal degradation by proteases. Such attempts included formulations with protease inhibitors and/or absorption enhancers or mucoadhesive systems. Still, oral delivery of insulin remains an unmet need [3]. As a result of this, colonic delivery and release of insulin has gained increasing interest by researchers because of the longer transit time, prolonged localization of insulin on the gut mucosa,

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lower levels of proteases in the colon or mucosal P-glycoprotein and greater responsiveness to permeation enhancers compared to the more proximal regions of the gastrointestinal tract. Such features would point to the colon as an interesting target for insulin oral delivery but, so far, an efficient drug delivery system for this propose is still missing [3,6,7–9].

A few attempts to improve the oral delivery of insulin by means of nanoparticle-based vectors have been reported [10–12]. Several nanoparticle (NP) types have been designed to protect biological drugs, including insulin, against chemical and enzymatic degradation and to enhance the intestinal absorption through paracellular and transcellular pathways [10,11]. Structural characteristics of nanoparticles, including size and surface charge, have been shown to influence the insulin absorption by the enterocytes. In general, small particles, provided with a positive charge are absorbed more efficiently through the intestinal epithelium. This is due to the interaction of NPs bearing positive charges with mucin residues that are negatively charged at physiological pH. The consequent prolonged residence time and increased concentration gradient at the surface of the intestinal mucosa might therefore promote protein absorption [3,11]. *Ex-vivo* and *in vivo* studies have also proven the potential of colloidal nanoparticles in increasing insulin absorption throughout the colonic region, but the lack of an appropriate delivery system that ensures their safe transit through the upper gastrointestinal tract strongly limits their usefulness [12,13]. Therefore, a solid dosage form, including pellets and tablets, which could host drug-loaded nanoparticles and possibly undergo a subsequent coating process, might represent a valuable strategy to enhance stability and provide release versatility of these colloidal systems administered via the oral route [6].

The objective of the present study was to prepare, characterize and evaluate both *in vitro* and *in vivo,* a novel nanoformulated, multiple-unit colon release system, *i.e.* coated pellets, as a possible oral nanocarrier for insulin. The novelty of this approach was the evaluation of the synergistic effect of colon release, mucoadhesive nanoparticles and the presence of a permeation enhancer, sodium glycocholate. The proposed multi-approach strategy combines the well-known benefits of this multiple-unit formulation in terms of reproducible transit time through the gastrointestinal tract, the consequent absorption pattern with the advantages of colloidal nanoparticles [14]. For this purpose, a recently proposed three-layer release technology platform was applied, consisting of a flexible film composed of a neutral polymethacrylate Eudragit® NE and a superdisintegrant sodium starch glycolate Explotab®, added as a pore former, applied to a hydroxypropyl methylcellulose (HPMC) coating of reduced thickness in order to improve the efficiency of the erodible layer in delaying the drug liberation [14–16]. An outer gastroresistant layer was also added in order to neutralize the variable residence time in the stomach of the coated dosage form and allow its activation only following the entry into the duodenum. This time-dependent relies on the relative consistency of short intestinal transit time, the subsequent colon targeting and favoring the intestinal absorption of insulin at that level [17].

2. Materials and methods

2.1. Materials

Bovine insulin (MW 5734 Da), polyethylene imine (MW 750 kDa), dextran sulfate (MW > 500 kDa), zinc sulfate, streptozotocin (STZ) and cellulose ester dialysis membrane tubing with a molecular weight cut-off (MWCO) of 1.000.000 Da (Spectra/Por® Biotech CE) were purchased from Sigma-Aldrich (St Louis, MO, US). All chemicals were used as received without further purification. Lactose was obtained from Prodotti Gianni (Milan, Italy). Microcrystalline cellulose co-processed with sodium carboxymethyl cellulose (Avicel® CL611) and hydroxypropyl methyl cellulose acetate succinate (Aqoat® LG, HPMCAS) were gifts from FMC Europe (Brussels, Belgium, distributed by IMCD Italia, Milan, Italy) and from Shin-Etsu (Tokyo, Japan, distributed by Seppic, Milan, Italy), respectively. Hydroxypropyl methylcellulose (Methocel® E50, HPMC) was kindly donated by Colorcon (Milano, Italy). Poly(ethylacrylate, methylmethacrylate) (2:1 monomer molar ratio) as 30% V:w aqueous dispersion (Eudragit® NE 30 D) of Evonik Röhm (Darmstadt, Germany) was a kind gift of Rofarma (Gaggiano, Italy). Polyethylene glycol (PEG 400) and size 4 hard-gelatin capsules were purchased from ACEF (Fiorenzuola D'Arda, Italy). Sodium glycocholate (NaGly) was obtained from and Tokyo Chemical Industry (Tokyo, Japan). Sodium starch glycolate (Explotab® CLV) was a gift from JRS Rettenmaier Italia (Castenedolo, Italy).

2.2. Synthesis of insulin-containing nanoparticles (nanoformulated insulin, NI)

22.1 mL of insulin solution (10 mg/mL in 0.01 M HCl), 10.8 mL of a 10% w/V dextran sulfate (DS) solution and 18.0 mL of 10 mM tris buffer, pH 9, were added under stirring (500 rpm) to 12.6 mL of polyethylene imine (PEI) solution (25% w/V). Afterwards, the mixture was heated at 40 °C and maintained under stirring because of its high viscosity and 7.8 mL zinc sulfate solution (2 M) were added, dropwise. As a result of the addition of the stabilizer, the formation of the nanoparticles took place and the viscosity of the solution decreased. The product was stirred for 15 min at 40 °C. The product was finally dialyzed in Milli-Q® water with cellulose ester dialysis membrane tubing with a molecular weight cut-off (MWCO) of 1.000.000 Da.

2.3. Synthesis of placebo nanoparticles (NPs)

22.1 mL of HCl 0.01 M, 10.8 mL of a 10% w/V DS solution and 18.0 mL of 10 mM tris buffer, pH 9, were added under stirring (500 rpm) to 12.6 mL of polyethylene imine (PEI) solution (25% w/V) and then treated as described above.

2.4. Nanoparticle characterization

2.4.1. Dynamic light scattering (DLS) and zeta potential measurements

The mean diameter and surface charge of the nanoparticles were assessed with a Zetasizer Nano ZS ZEN3600 from Malvern Instruments Ltd (Worcestershire, United Kingdom) operating at a light source wavelength of 633 nm and a fixed scattering angle of 173°. The sample concentration was chosen to keep attenuator values between 7 and 9. The refractive index of material was 1.524. The measurements were performed in triplicate, after dilution of the nanoparticles respectively with MilliQ® water and aqueous solution of sodium chloride (1 mM).

2.4.2. Transmission electron microscopy (TEM) analysis

Nanoparticles were visualized using 120 keV TEM (Jeol 1010, Tokyo, Japan). Two microliters of the sample, along with 2% w:V uranyl acetate solution, were deposited onto a piece of ultrathin 200-mesh copper grid (Ted-pella, Redding, CA, US) and left to dry in air before examination by TEM.

2.4.3. Determination of the entrapment efficiency (EE%) of insulin into the nanoparticles

The amount of insulin encapsulated into the nanoparticles was determined suspending the NI, corresponding to 30 μg/mL theoretical insulin concentration, in 0.05 M HCl and centrifuging at 12000 rpm for 20 min (ScanSpeed 1730R, Labogene, Lynge,