European Journal of Pharmaceutics and Biopharmaceutics 123 (2018) 84–94

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Retrograded starch/pectin coated gellan gum-microparticles for oral administration of insulin: A technological platform for protection against enzymatic degradation and improvement of intestinal permeability

Andréia B. Meneguin[a,b,1], Eric Beyssac[c], Ghislain Garrait[c], Hassana Hsein[c], Beatriz S.F. Cury[a,*]

[a] Department of Drugs and Pharmaceuticals, School of Pharmaceutical Sciences, São Paulo State University-UNESP, 14800-903 Araraquara, SP, Brazil
[b] Interdisciplinary Laboratory of Advanced Materials, Centro de Ciências da Natureza – CNN, Universidade Federal do Piauí – UFPI, 64049-550 Teresina, PI, Brazil[1]
[c] Université Clermont Auvergne, INRA, UMR MEDIS, F-63000 Clermont-Ferrand, France

ARTICLE INFO

Keywords:
Insulin
Gellan gum
Retrograded starch
Colon-specific delivery
Microparticles

ABSTRACT

Gellan gum microparticles coated with colon-specific films based on retrograded starch and pectin was developed for enhancing the oral release of insulin (INS). The system developed promoted an impressive protection of INS (80%) after 120 min of incubation with trypsin and alpha-chymotrypsin, while only 3% of free INS remained intact after the same time, possibility due to the calcium chelating activity of the polymers in inhibiting the proteolytic activity. *In vitro* INS release in media simulating the gastrointestinal portions revealed a pH-dependent behavior, as well as the significance of the coating in lowering the release rates in relation to their counterparts. The permeability of INS on Caco-2 cells monolayers and excised rat intestine were significantly improved, mainly due to the influence of the anionic polymers on tight junctions opening, along with the excellent mucoadhesive properties of the gellan gum. All these features together contributed greatly to the hypoglycemic effect observed after the oral administration of the INS-loaded MP in diabetic rats, with reduction of up to 51% of blood glucose levels. The important findings of this work should contribute to the advances about the search of alternatives for oral administration of INS.

1. Introduction

After approval of recombinant insulin in 1982, the pharmaceutical industry has been revolutionized with the exponential increase of the therapeutic proteins approved by the FDA [1], which correspond to 50% of new drugs approved, reaching today about 200 new marketed biologics-based medicines [2]. The successful use of the biotherapeutics, mainly in the treatment of inflammatory, autoimmune, cardiovascular, metabolic diseases and in several types of cancer, is because of their numerous complex functions performed in the organism with greater specificity of action and safety over conventional chemical drugs, ensuring optimized therapeutic effects and reduced side effects [1,3].

It is worth watching that clinical efficacy of protein therapeutics is also a result of the pathway by which these biomolecules are administered, which in most cases occurs *via* parenteral route. A classic example of this is the treatment of diabetes mellitus (type-I and advanced cases of type-II), which over the decades has been carried out through daily subcutaneous injections of exogenous insulin (INS). Although the parenteral route of administration is quite effective in terms of bioavailability, the invasive routes cause several discomforts, including pain, local infection, lipoatrophy and psychological problems, reducing the compliance of the patient to the treatment [4,5].

In this regard, several strategies had been evaluated in order to find alternative routes for INS administration, such as the development of systems for nasal [6], rectal [7], pulmonary [8] and transdermal [9] routes. However, oral administration is considered the most convenient owing to its non-invasive nature, safety and comfort of the self-administration. In addition, INS is one of the few drugs benefited by the first hepatic bypass, *i.e.*, after its absorption by the intestinal epithelium, INS reaches the liver *via* the hepatic portal vein and inhibits directly the glucose production, mimicking the natural secretion of INS by islets of Langerhans. This effect cannot be reached by parenteral administration, as only 20% of the injected INS reaches the liver [10,11].

Unfortunately, the oral administration of proteins/peptides is limited by their intrinsic properties (high molecular weight, hydrophilic,

* Corresponding author.
E-mail addresses: abagliottim@hotmail.com (A.B. Meneguin), eric.beyssac@uca.fr (E. Beyssac), ghislain.garrait@uca.fr (G. Garrait), hassana_hsein@hotmail.com (H. Hsein), curybsf@fcfar.unesp.br (B.S.F. Cury).
[1] Present address.

https://doi.org/10.1016/j.ejpb.2017.11.012
Received 2 June 2017; Received in revised form 6 October 2017; Accepted 23 November 2017
Available online 23 November 2017
0939-6411/

surface charges and instability) and mainly by the harsh barriers of the gastrointestinal tract (GIT). Thus, INS undergoes conformational change/denaturation when in contact with the extremely acidic stomach fluid, followed by enzymatic digestion in the small intestine by the action of proteolytic enzymes, mainly trypsin and alpha-chymotrypsin, so that less than 0.1% of the INS dosage reaches the bloodstream in intact form [12]. Furthermore, the intestinal epithelium itself acts as a physical barrier against the permeation of the INS molecules, which in turn have high hydrophilicity and molecular weight, contributing to its reduced intestinal permeability [13].

In this work, we present a multi-strategy technological platform to enhance the oral bioavailability of INS, a BSC class III drug. The microencapsulation of INS seems to be a rational strategy for its protection against acid denaturation and enzymatic digestion, since its effective entrapment within the polymeric matrix should restrict the contact with H^+ ions from the gastric juice as well as with the proteolytic enzymes [14,15]. The gellan gum (GG) – a linear anionic polysaccharide and soluble in water – can be successfully used as encapsulating polymer because of its ability to form microparticles (MP) in the presence of multivalent cations [16,17]. The mucoadhesive properties of GG should also contribute to immobilization of MP in a target organ by a more intimate contact with intestinal epithelium (main absorption site) for extended periods of time, which favors the local concentration gradient, bypassing the drawbacks of intestinal permeability [18,19].

Several papers have shown that the colon is a favorable site for absorption of proteins/peptides [20–22], owing to its peculiar characteristics, such as pH near to neutrality, longer transit time and reduced activity of the proteolytic enzymes [23]. In this case, the colon-specific release of INS can be achieved by coating the INS-loaded MP with resistant starch/pectin (RS/P) films, which were previously developed and characterized in an earlier study published [24]. This is because the three-dimensional structure of such RS undergoes conformational alteration after being hydrothermally modified (retrogradation process) to a more ordered state in which the peptide bonds are protected from enzymatic attacks, conferring to this material high protective ability against digestion in the upper portions of the GIT. Upon reaching the colon, RS is specifically digested by the enzymes produced by the colonic microbiota, allowing the colon-specific release of INS [25]. It is important to highlight that the RS/P film coating can also exert an important role in the control of drug release kinetics.

Regarding the natural anionic polymers mentioned herein, there are important reports in the literature of their use for enhancing the absorption [26,27] as well as to inhibit the enzymatic digestion [28], which should represent an important alternative to conventional absorption enhancers and protease inhibitors, since many of them are toxic and act in a non-specific way. The aforementioned activities may be related to the calcium chelating activity, which plays an important role in the maintenance of intercellular contact, as well as being fundamental in the thermodynamic stability of the proteases [29,30].

On the basis of such premises, the aim of the present work was to encapsulate INS within mucoadhesive GG MP coated with RS/P films, as a promising strategy to overcome the problems of enzyme degradation and intestinal permeability, besides successfully releasing the drug in a specific gastrointestinal area (colonic delivery). In order to prove the interest of such formulation, it was necessary to evaluate (i) the protective effect of coated and uncoated MP against enzymatic degradation, (ii) the impact on permeability of INS both in Caco-2 cells and excised rat intestine, (iii) the release profiles of INS in dissolution media simulating the gastrointestinal tract conditions using flow-through cell apparatus (USP Apparatus 4) and (iv) the hypoglycemic effects in diabetic rats after oral administration.

2. Materials and methods

2.1. Materials

Pectin (type LM-5206CS – DE < 50%) and gellan gum (type Kelcogel® CGLA) were kindly provided by CP Kelco (Limeira, SP, Brazil). High amylose starch (HAS; type Hylon VII – 68% amylose) was a gift of the National Starch & Chemical (New Jersey, USA). Glycerin (99.5%), aluminum chloride ($AlCl_3$) and sodium chloride was purchased from Vetec (Duque de Caxias, Brazil). Human insulin solution (Novolin R 100 IU/mL) was obtained from Novo Nordisk A/S. Acetonitrile, trifluoroacetic acid (TFA), dimethylformamide (DMF), hydrochloric acid 37% v/v (HCl), phosphoric acid extra pure (H_3PO_4), sulfate sodium anhydre (Na_2SO_4), trypsin-EDTA, phosphate buffered saline (PBS), sodium hydroxide (NaOH), Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum, vitamins, nonessential amino acids, L-glutamine, antibiotic solution (penicillin 100 IU/mL, streptomycin 10 mg/mL, and amphotericin B 25 μg/mL) and Hank's balanced salt solution (HBSS) were purchased from Fisher Scientific (Illkirch, France). Trypsin (11,800 U/mg) and α-chymotrypsin (59.3 U/mg) from bovine pancreas, N-benzoyl DL-arginine p-nitroanilide (BAPNA), N-benzoyl-L-tyrosine p-nitroanilide (BTPNA), Krebs Henseleit modified buffer and alloxan were purchased by Sigma Aldrich (Saint Quentin Fallavier, France). Transwell® Permeable Supports (Insert diameter 12 mm, 12 well, pore size 0.45 μm, insert membrane growth area 1.12 cm^2) and T-75 cm^2 flasks were purchased from Corning (Lowell, USA). Ketamine (Imalgene 1000®) was obtained from Merial laboratory, France.

2.2. Retrogradation of HAS mixed with pectin

The process of HAS retrogradation was carried out in two steps using alternating thermal cycles according to Meneguin et al. [24]. Briefly, aqueous dispersion of HAS at 5% (w/v) was gelatinized by autoclaving at 121 °C (120 min) and then mixed with pectin (5% w/v) at 1:1 ratio. After that, the dispersions were submitted to the retrogradation process by storage at 4 °C and 30 °C for 16 days (two days at each temperature) and after labeled as RS/P5.0 (patent no. BR 10 2016 028675 1).

2.3. Preparation of tested polymer dispersions

GG dispersions at 1.5 and 2.0% (w/v) (GG1.5 and GG2.0, respectively) were prepared by dispersing the polymer in heated water (60 °C) under constant magnetic stirring until complete homogenization.

For the further tests "Protective effect of MP against INS degradation" (Section 2.8.3) and "Transport experiments on Caco-2 cells" (Section 2.10.4), INS was added to all dispersions after the temperature cooled down until 37 °C, under mild magnetic stirring for 1 h, to a final concentration of 200 ug/mL. These INS-loaded dispersions were identified by adding the INS suffixes, as GG1.5-INS, GG2.0-INS, RS/P5.0-INS.

2.4. Microparticles preparation

MP were prepared by the ionotropic gelation technique. Briefly, GG aqueous dispersions (pH 4.5) at 1.5 or 2.0 (w/v) prepared according to Section 2.3 were dropped through 23G-gauze flat-tipped needle (25 × 0.6 mm) into a cooled solution of aluminum chloride (3 or 5%; *w/v*), containing INS solution (6.5% (*v/v*)) and respecting 1:1.3 GG dispersion:crosslinker ratio. MP were kept under magnetic stirring for 24 h to achieve maximum crosslinking and INS loading, followed by filtration and several rinsing with distilled water. After that, MP were dried at 25 °C for 24 h in an oven with forced air circulation and stored in a desiccator. Samples without INS were prepared as control (patent no. BR 10 2016 028675 1).