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Development of a Gas Empowered Drug Delivery system for peptide delivery in the small intestine

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ABSTRACT

The aim of this investigation was to design a novel Gas Empowered Drug Delivery (GEDD) system for CO_2 forced transport of peptide drugs together with mucoadhesive polymers to the surface of the small intestine. The GEDD effect of the core tablet was achieved using CO_2 gas to push insulin together with the mucoadhesive excipients poly(ethyleneoxide) (PEO) and the permeation enhancer trimethyl chitosan (TMC) to the surface of the small intestine. The in-vitro insulin release showed that almost 100% of the insulin was released from enterically coated tablets within 30 min at pH 6.8. The designed GEDD system was shown to increase the insulin transport by approximately 7 times in comparison with the free insulin across sheep's intestine ex-vivo. Three different peroral formulations were administered to male rabbits: F1 containing no TMC or PEO, F2 containing PEO but no TMC and F3 containing both PEO and TMC. The administrations of insulin using the formulation F1 resulted in a low FR value of 0.2%±0.1%, while the formulations F2 and F3 resulted in a much higher FR values of 0.6±0.2% and 1.1%±0.4%, respectively. Hence, the insulin permeation achieved by the GEDD system is primarily due to the enhancing effect of TMC and the mucoadhesive properties of PEO both of which synergistically increase the bioavailability of insulin.

1. Introduction

In the past decade, a large number of endogenous peptides and protein drugs are produced in biotechnological companies for the treatment of chronic diseases. The majority of these drugs are administered by parenteral injection which is inconvenient, time and money consuming as well as dangerous. Oral drug delivery is the most convenient way for self administration of drugs, allowing a wide range of dosage adjustments [1]. Hence, a lot of research is focused on design and development of novel oral drug delivery systems for both hydrophilic, large macromolecules such as peptide and protein drugs and also small hydrophilic drug molecules with poor bioavailability.

Effective oral delivery of therapeutic peptides and proteins to the small intestine poses a great challenge in drug delivery system design. The harsh environment of the stomach, the presence of proteolytic enzymes in the small intestine, the hydrophilicity of the peptide molecules and its large molecular size as well as the poor membrane permeability have led to an intense investigation for site- specific drug delivery systems [2–4]. In order to overcome the above obstacles, the ideal oral delivery system must release its contents pH dependently only at the optimal target region, remain in the optimal site long enough for the complete peptide and protein release to be absorbed across the intestinal epithelium, and have a reproducible therapeutic effect. Thus, site specific delivery is required to deploy the peptides and proteins intact to specifically targeted parts of the body through a platform that can control their release by means of physiological or chemical triggers [5–7].

A number of sophisticated oral novel delivery systems have been developed for the delivery of peptides and proteins [8]. Most of these delivery systems, however, are only designed to release the hydrophilic macromolecule at the desired site of action but do not take into account appropriate mechanisms to also facilitate absorption of the drug molecule which in most cases is then enzymatically degraded in the small intestine [9].

In order to actively improve drug absorption a lot of focus was given to the mucoadhesive polymers for oral delivery of peptide drugs. Mucoadhesive polymers are hydrophilic macromolecules with numerous hydrogen bond forming groups such as carboxyl, hydroxyl, amide

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and amine groups that need to be hydrated for swelling and to act as a mucoadhesive polymers on the mucus linings of the gut. Suitable mucoadhesive polymers like chitosans and their quaternary derivatives form intermolecular complexes with the glycoproteins of the mucus linings to form a stable adhesive layer with the mucus. This adhesion can further assist in the absorption of peptide drugs across the mucosal membrane of the gut by reversibly opening of the tight-junctions [10–12].

Chitosan, a biocompatible and biodegradable polymer has been used as an oral drug delivery vehicle. However, chitosan can be used as an enhancer only in the proximal part of the intestine where the pH is close to its p*K*a value of 6.5. Quaternized derivatives of chitosan were synthesized and shown in contrast to chitosan to be drastically more soluble in neutral and alkaline environments of the intestine and more useful for drug delivery and absorption across the intestinal epithelium of the jejunum and ileum [13,14]. The permeation enhancing properties of these chitosan derivatives have been attributed to their ion pair interactions with the tight-junctions and cellular membrane components to increase the paracellular permeation of hydrophilic compounds [15]. The polymer charge density, determined by the substitution degree is a key factor in obtaining both the mucoadhesion and penetration enhancement towards the intestinal epithelium [16,17]. Among chitosan derivatives, TMC was shown to have the most permeation enhancing effect. Hence, TMC was synthesized from low molecular weight chitosan with a degree of quaternization of approximately 50±5% which has been shown to have the highest penetration enhancing effect across the intestinal epithelium in vitro and in vivo [18].

However, the use of mucoadhesive polymers used as delivery platforms in the micro- and nanosize poses the problem that after the release of these mucoadhesive units they firstly have to swell before they can fully exert their mucoadhesive properties. Already during this swelling process the mucoadhesive sites can be de-activated by soluble mucins amply available in the intestinal fluids rendering in systems which are unable both to adhere to the mucous linings of the gut wall and to trigger the opening of the tight junctions. This holds especially for big gut lumina as those of the pig and humans [19,20]. In order to overcome this drawback, Dorkoosh et al. have developed a time- and site-controlled delivery system that kept the drug mechanically attached to the intestinal site of absorption. They have used superporous hydrogel (SPH) and SPH composite (SPHc) polymers that were able to swell very quickly due to their highly porous structure and incorporated in these conveyor systems suitable delivery systems for the drug octreotide to get a desirable time-controlled release profile, required for absorption of peptides and proteins. They succeeded in getting high absolute bioavailabilities (F=8–16%) depending on the type of delivery system in an in vivo study in pigs [21–24]. However these systems were not suitable for industrial mass production.

In this study, an easier to produce system namely a CO_2 empowered drug delivery (GEDD) system has been designed to quickly release insulin and the mucoadhesive polymers (polyethylene oxide) (PEO) and trimethyl chitosan (TMC) and to push them by the CO_2 bubbles to the mucous linings without losing their mucoadhesiveness and potency to open the tight junctions.

The delivery system is enterically coated to release its contents in the proximal part of the small intestine where the CO_2 gas directs the mucoadhesive PEO polymer to attach to the mucosa of the small intestine. Once attached, the permeation enhancer, TMC, will also bind to the mucosal surface and trigger the opening of the tight junctions of the epithelial cells of the small intestine and open the paracellular pathway to facilitate the transport of the insulin across the mucosal membrane.

Robinson et al. have done studies using CO_2 as absorption enhancing agent in the ex vivo intestine of rabbit and mouse and showed that the direct bubbling of CO_2 can enhance drug permeability by reversibly altering the paracellular pathway [25]. However in the newly designed GEDD systems described here, CO_2 is not primarily used as a penetration enhancer but as pushing agent of the drug containing excipients to the absorbing surface.

Hence, the aim of this study was to investigate 1) the mucoadhesive and permeation enhancing effect of the novel delivery system 2) the in-vitro insulin release from the delivery system in the two different milieus of stomach and intestine 3) to evaluate the effectiveness of the GEDD system as permeation inducer of insulin in the sheep's intestine in ex vivo studies and 4) to determine the bioavailability of peroral insulin using GEDD delivery system in an in vivo study in rabbits.

2. Materials and methods

2.1. Materials

Chito Clear® chitosan (viscosity 1% w/v solution, 22 mPa s) was purchased from Primex, Iceland. Human insulin was a generous gift from Exir Pharmaceutical Company (Lorestan, Iran). The Polyethylene oxide (Mw 900,000) was a gift from (Dow Chemicals, U.K.). TMC with the degree of substitution of 50±5% was synthesized in our laboratory as described previously [26]. Cellulose acetate phthalate (CAP) was purchased from Trans Medica (Germany), Ac-Di-Sol was purchased from SMF (Netherlands). All the other materials were of pharmaceutical and analytical grades and used as received.

2.2. Formulation and design of the GEDD system

The delivery system was prepared based on a 2^3 factorial design. The variables were the percent polyethylene oxide (PEO) as mucoadhesive polymer, percent of citric acid and Na-bicarbonate (for CO_2 gas production), and Ac-Di-Sol as super-disintegrant. The response was the CO_2 gas production, tablet disintegration time as well as a mucoadhesive mass response. The variables were set as low and high values of 50% and 70%, 2.5% and 5.0% and 5.0% and 10% of the total weight for the amount of acid-base, Ac-Di-Sol and the PEO, respectively. As it was impossible to quantify the response of the CO_2 gas produced, designated (+) was used to evaluate the formulations 1–8. Accordingly one plus (+) corresponds to a low response and two (++) corresponds to a high response. The disintegration response (amount of Ac-Di-Sol) was evaluated by the disintegration time of the tablets in PBS of pH 6.8 at 37 °C. Finally, the mucoadhesive response was measured by the amount of work (N) required to detach the dosage form from the sheep's intestinal mucosa in vitro as described in Section 2.5. According to the results obtained from the factorial design experiment, the following formulation was used for further studies: (46.7% citric acid, 23.3% Na-bicarbonate, 5% Ac- Di- Sol, 5% Avicel PH102, 10% PEO, 3.0% lactose 80, 1.5% human insulin, 0.5% TMC, and 5.0% Na-benzoate as lubricant).

In a lab-scale formulation, absolute alcohol was used for a wet granulation with citric acid, Na- bicarbonate and lactose. TMC, PEO, Avicel, insulin and the Na-benzoate were then added step-wise to the obtained granules and mixed well using manual mixing. The granules were then pressed to tablets using a single, lab scale press machine with a round, concave punch #7 at a 150 mg±7.5% weight and hardness of 4–6 Kpa. Content uniformity of the tablets was determined according to the standard method of USP 30 where 10 tablets were used to assay the amount of insulin. The RSD of the tablets weights was below 6%. The percent of insulin was calculated from the following equation: Ru/Rs×100=% Insulin in each tablet.

Ru and Rs are the respective areas under the curve for the test and standard samples (data not presented).

2.3. Preparation of enteric coated tablets

Once the tablets were pressed, they were enterically coated with a 3.0 mg sub-coat of a 3% solution of polyvinylpyrrolidone (PVP) K30 in absolute isopropanol and subsequently by 11 mg of a 6% CAP solution containing diethyl phthalate as plasticizer per single tablet. The PVP