Oral Delivery System for Two-pulse Colonic Release of Protein Drugs and Protease Inhibitor/Absorption Enhancer Compounds

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ABSTRACT: It is well known that the intestinal stability and absorption of protein drugs are improved when enzyme inhibitors/permeation enhancers are coadministered. Recently, it was hypothesized that an increased effectiveness of these adjuvants might be achieved by timing their release prior to that of the protein, so that a more favorable environment would be established in advance. Therefore, an oral system was proposed for two-pulse colonic release of insulin and the protease inhibitor camostat mesilate/absorption enhancer sodium glycocholate. The device consisted of a drug-containing core, an inner swellable/erodible low-viscosity hydroxypropyl methylcellulose (HPMC) coating, an intermediate adjuvant layer, and an additional outer HPMC coating. HPMC coats and camostat mesilate/sodium glycocholate films with differing thicknesses were applied to immediate-release tablet cores by aqueous spray coating. The obtained units were characterized for weight, thickness, breaking force, and release performance. All systems showed satisfactory technological properties and the pursued pulsatile delivery behavior, with programmable delay phases preceding inhibitor/enhancer release and elapsing between inhibitor/enhancer and protein release, respectively. Indeed, both lag times linearly correlated with the relevant HPMC coating level. The system was thus proven suitable for yielding two-pulse release profiles, in which lag phases could be modulated to provide convenient concentration patterns for proteins and adjuvants. © 2011 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 100:3251–3259, 2011

Keywords: coating; colonic drug delivery; formulation; insulin; *in vitro* release; oral drug delivery; peptide delivery; pulsatile release

INTRODUCTION

Currently, protein and peptide drugs are widely used in therapy at present as a consequence of the recent advances in biotechnology and increased knowledge of the etiopathology of many disorders. Noninvasive delivery of these molecules, however, remains a major challenge, particularly with regard to the oral route. As a matter of fact, the number of peroral biotechnological products currently available on the market is quite limited.[1,2] Proteins are generally hydrophilic compounds with a high molecular weight and poor permeability through the gastrointestinal mucosa. In addition, their stability is often impaired at extreme pH values (e.g., in the stomach) and in the presence of proteolytic enzymes. In order to overcome the limitations that hinder oral protein delivery, numerous formulation strategies have been investigated. In this respect, release into the colon has been proposed due to a lower presence of luminal and membrane-associated proteases as well as reduced fluid volume, which would allow higher concentrations to be reached for important functional adjuvants such as absorption enhancers and protease inhibitors.[1,3–5]

More recently, an increased attention has been focused on the adjuvant release pattern and its possible influence on the macromolecule bioavailability. In particular, it has been suggested that the intestinal absorption of peptides and proteins might positively be affected by a concurrent release of the protease inhibitor and/or absorption enhancer.[5] The stability of the protein could indeed be improved as the inhibitor would prevent its enzymatic degradation by acting as a "guard molecule" prior to absorption. Moreover,

Additional Supporting Information may be found in the online version of this article. Supporting Information

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the permeation through the intestinal mucosa would be promoted by a transient modification of its barrier characteristics occurring at the time of drug liberation.

On the other hand, it has recently been hypothesized that the possibility of timing the adjuvant release to occur prior to that of the protein could be advantageous as a more favorable environment might thus be established in advance.[6,7] In particular, a lag time of approximately 15–20 min has been assumed to be necessary for the adjuvant to inactivate the proteolytic enzymes and modify the permeability characteristics of the intestinal epithelium. An oral device based on an acrylic superporous hydrogel matrix protecting an octreotide-containing core was accordingly described.[8] As a result of the increase in volume undergone upon contact with physiological fluids, the hydrogel unit would mechanically adhere to the small intestinal wall and defer drug release while exerting protease inhibition and permeation enhancement actions. A notable bioavailability increase was obtained when such a system was tested in an animal study.

Subsequently, an oral time-dependent colon delivery system that was able to modulate the release of functional adjuvants with respect to drugs susceptible to intestinal degradation and/or poor mucosa permeation (e.g., proteins and peptides) was designed. For this purpose, a previously described swelling/erosion-based colonic release platform (Chronotopic™), which was already proven suitable for the conveyance of bovine insulin, was adapted to match both the aforementioned hypotheses concerning the adjuvant release mode.[9–11] The system consisted of a drug-containing core and a water-swellable polymeric coating (low-viscosity hydroxypropyl methylcellulose, HPMC) responsible for a programmable delay preceding the onset of release. According to the time-dependent formulation approach, which exploits the relatively reproducible small intestinal transit time of dosage forms, an outer enteric film was included to prevent the site of drug liberation from being affected by highly variable gastric residence.[12]

When intended for a concomitant release of the protein drug and the relevant adjuvants, the system was based on a disintegrating core tablet in which such compounds were enclosed.[13] The pursued concurrent delayed release of insulin and the protease inhibitor camostat mesilate as well as absorption enhancer sodium glycocholate was thereby obtained following assessment of the mutual compatibility.

Alternatively, it was proposed to insert a protease inhibitor/absorption enhancer interlayer within the HPMC coating so that the adjuvant could be delivered beforehand.[14]

On the basis of these premises, the present work was focused on the preparation and evaluation of multilayered formulations able to yield two-pulse release patterns of insulin and camostat mesilate or sodium glycocholate, thus ideally complying with the benefits that were assumed to result from a preventive action of the adjuvant at the site of protein delivery.

MATERIALS AND METHODS

Materials

Following materials were used in the present study: bovine insulin (Sigma–Aldrich, St. Louis, Missouri), camostat mesilate (Daito Corporation, Tokyo, Japan), HPMC (Methocel® E50; Colorcon, Gallarate, Italy), magnesium stearate (Carlo Erba Reagenti, Milan, Italy), microcrystalline cellulose (Avicel® PH200; FMC Europe, Brussels, Belgium), polyethylene glycol 400 (PEG 400; A.C.E.F., Piacenza, Italy), poly(methacrylic acid-*co*-ethyl acrylate) dispersion 30% (Eudragit® L 30D-55; Röhm Pharma, Darmstadt, Germany), sodium glycocholate (Sigma–Aldrich), sodium starch glycolate (Explotab® CLV; Mendell, Patterson, New York), talc (Carlo Erba Reagenti, Milan, Italy), and triethyl citrate (TEC; Fluka Chemie, Buchs, Switzerland).

Preparation and Characterization of the Delivery Systems

An exactly weighed amount of bovine insulin was mixed in a mortar with the core excipients. The final percent composition (w/w) of the powder mixture was 2.5 bovine insulin, 92.5 Avicel® PH200, 4.5 Explotab® CLV, and 0.5 magnesium stearate. Compression was carried out in a rotary machine (AM-8S; Officine Ronchi, Milan, Italy) equipped with 5 mm diameter concave punches at an approximate compression force of 400 k_p.

Tablets were checked for weight, height, and diameter (digital micrometer CD-15D calibrated at 0.01 mm; Mitutoyo Corporation, Kawasaki, Japan); breaking force (crushing tester TBH28; Erweka, Heusenstamm, Germany); friability (friabilometer TA3R; Erweka); and disintegration time (three-position USP 32 disintegration apparatus DT3; Sotax AG, Basel, Switzerland). All measurements were performed in 20 replicates except for disintegration time (n=6).

Tablet cores were first coated in a top-spray fluid bed (Uniglatt®; Glatt GmbH, Binzen, Germany) to increasing weight gains (w.g.; 13%, 25%, 50%, and 100%) with an 8% (w/v) Methocel® E50–0.8% (w/v) PEG 400 aqueous solution according to previously established operating conditions.[9] The coating solution was prepared by dissolving PEG 400 in deionized water and subsequently adding the HPMC powder under magnetic stirring at 80°C. The resulting dispersion was then stored at 4°C for 24 h to enable