Preliminary In Vivo Evaluation of an Oral Multiple-Unit Formulation for Colonic Delivery of Insulin

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ABSTRACT SUMMARY

A multiple-unit formulation for time-dependent colonic release of insulin was prepared and evaluated. The system comprised a minitablet core containing the protein and a permeation enhancer, an internal swellable/erodible layer based on low-viscosity hydroxypropyl methylcellulose (HPMC), an intermediate Eudragit®NE film including a superdisintegrant (sodium starch glycolate), able to slow down water penetration into the underlying functional layer, and an outermost enteric coat intended to overcome the issue of variable gastric residence. When this system was administered to diabetic rats, a peak in insulin plasma concentrations was observed 6 h post-dose along with a drop in glycaemia. Both could coincide, based on rat gastrointestinal transit times, with the system being positioned in the ileo-colonic region.

INTRODUCTION

An oral pulsatile delivery system (Chronotopic™), based on a low-viscosity hydroxypropyl methylcellulose (HPMC) erodeable coating, was recently proposed in a multiple-unit configuration [1]. This involved the application of an insoluble, flexible film composed of the neutral polymethacrylate Eudragit®NE and the superdisintegrant sodium starch glycolate, added to act as a pore former, to the functional HPMC layer. The Eudragit®NE/sodium starch glycolate film was intended to improve the efficiency of the system in delaying the drug liberation without altering the typical release pattern it shows when provided with the HPMC coating only. The resulting two-layer formulation was also proposed for time-dependent colon delivery of insulin in view of the advantages that the large bowel has been reported to offer as a release site for protein drugs, such as a lower gastric residence. When this system was administered to diabetic rats, a peak in insulin plasma concentrations was observed 6 h post-dose along with a drop in glycaemia. Both could coincide, based on rat gastrointestinal transit times, with the system being positioned in the ileo-colonic region.

EXPERIMENTAL METHODS

Materials: bovine insulin (Sigma-Aldrich, US-MO); copovidone (Kollidon®VA64, BASF, D); HPMC (Methocel®E50, Colorcon, I); hydroxypropyl methylcellulose acetate succinate (HPMCAS, Aqoat®AS-LG, Shin-Etsu, J, a gift from Seppic Italia, I); magnesium stearate (Carlo Erba Reagents, I); microcrystalline cellulose (Avicel®PH200, FMC Europe, B); poly(ethylacrylate, methylmethacrylate) aqueous dispersion (Eudragit®NE30D, Evonik Röhm, D, a gift from Roferma, I); polyethylene glycol (PEG 400, ACEF, I); sodium glycocholate (NaGly, Sigma-Aldrich, US-MO); sodium starch glycolate (Explotab® and Explotab®V17, Mendell, UK); streptozotocin (Sigma-Aldrich, US-MO). Methods: biconvex minitablets (2.5 mm) containing bovine insulin (0.4 mg) and NaGly (1:10) were coated with an 8% HPMC–0.8% PEG 400 aqueous solution by rotary fluid bed (GPG1.1, Glatt, D) up to a 250 µm coat thickness. HPMC-coated cores were afterwards coated by bottom-spray fluid bed with Eudragit®NE30D containing 20% w/w (on dry polymer) of Explotab®V17 up to a film thickness of 20 µm. Curing was carried out at 40°C for 24 h. The two-layer units were finally coated with a 6.0% HPMCAS hydroalcoholic (ethanol 75% w/w) solution in a ventilated coating pan (GS, I) up to an enteric coating level of 7.5 mg/cm². In vitro release testing (n=3) was performed by a modified USP 35 disintegration apparatus (DT3, Sotax, CH). HCl 0.1 M for 2 h and, subsequently, phosphate buffer pH 6.8 were used as the release media (37±1°C, 31 cycles/min, 160 ml) [5]. Insulin and NaGly were assayed by reverse phase-HPLC in fluid samples withdrawn at successive time points [6].

For the in vivo experiment, male Sprague Dowley rats, fed ad libitum and handled in accordance with the provisions of the European Economic Community Council Directive 86/209 (recognized and adopted by the Italian Government with the approval decree D.M. No. 230/95-B) and the NIH publication No. 85-23, revised in 1985, were treated with 65 mg/kg of streptozotocin subcutaneously administered. Animals with glycaemia of 400-500 mg/dl were subdivided into 3 groups and received orally samples of either the insulin-containing minitablet core or the coated formulation or, alternatively, an equal amount of the hormone in solution. At programmed time points, 50 µl of blood was collected by the tail vein and centrifuged. Plasma samples (10 µl) were diluted and glucose was assayed by the Trinder Kit (Sigma-Aldrich). Plasma samples (10 µl) were analyzed for insulin concentration by a human insulin-specific ELISA kit (Sigma-Aldrich).

RESULTS AND DISCUSSION

The multiple-unit colon delivery system under examination comprised an insulin- and NaGly-containing tableted core, an inner HPMC layer, aimed at delaying
drug release through a swelling/erosion-dependent mechanism, an intermediate Eudragit®SNE/sodium starch glycolate film, intended to prolong the duration of the lag phase as imparted by the underlying HPMC coat, and an outer gastroresistant film. The latter would protect the system while located in the stomach and prompt the lag time to be started in the duodenum, thus allowing the colon district to be targeted based on the relatively consistent small intestinal transit time of dosage forms [7]. This formulation was first evaluated for in vitro release: while no drug liberation was observed during the acid stage of the test, pulsatile release of insulin and NaGly took place after reproducible lag phases of comparable duration in simulated intestinal pH conditions (data not shown).

Administered to diabetic rats, the coated formulation brought about, as compared with an uncoated minitablet and a solution of the hormone, a steep and significant decrease in the blood glucose levels, which roughly coincided with a sharp rise in the plasma insulin concentration at approximately 6 h post-dose (Figures 1 and 2).

This indicate that the hormone would rapidly and effectively be absorbed after the pursued lag phase. From literature data on rat gastrointestinal transit it was inferred that the maximum insulin and minimum glucose concentrations would be related to an ileo-colonic location of the system [8,9].

CONCLUSION

A multiple-unit delivery system for time-dependent colonic release of insulin was prepared and evaluated. The proposed formulation exhibited the desired hypoglycaemic effect in diabetic rats.

REFERENCES


Figure 1. Mean blood glucose concentration vs. time profiles in diabetic rats following administration of coated systems, uncoated minitablets or a hormone solution.

Figure 2. Mean insulin plasma concentration vs. time profiles in diabetic rats following administration of coated systems, uncoated minitablets or a hormone solution.