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Improved oral absorption of exenatide using an original nanoencapsulation and microencapsulation approach

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ABSTRACT

Oral delivery is the most convenient and favorable route for chronic administration of peptides and proteins to patients. However, many obstacles are faced when developing such a delivery route. Nanoparticles (NPs) are among the leading innovative solutions for delivery of these drugs. Exenatide is a peptidic drug administered subcutaneously (SC) twice a day chronically as an add-on therapy for the worldwide pandemic disease, diabetes. Many attempts to develop oral nanocarriers for this drug have been unsuccessful due to the inability to retain this hydrophilic macromolecule under sink conditions or to find a suitable cross-linker which does not harm the chemical integrity of the peptide. In this study, we report about an original oral delivery solution based on a mixture of albumin and dextran NPs cross-linked using sodium trimetaphosphate (STMP). Moreover, we suggest a second defense line of gastro-resistant microparticles (MPs) composed of an appropriate ratio of Eudragit® L100-55 (Eudragit L) and hydroxypropylmethylcellulose (HPMC), for additional protection to these NPs presumably allowing them to be absorbed in the intestine intact. Our results demonstrate that such a system indeed improves the relative oral bioavailability of exenatide to a level of about 77% compared to subcutaneous injection due to the presence of dextran in the coating wall of the NPs which apparently promotes the lymphatic uptake in the enterocytes. This technology may be a milestone on the way to deliver other peptides and proteins orally.

1. Introduction

The majority of peptide and protein drugs are still administered by injection often causing pain-associated problems and major inconvenience to the patients [1,2]. Hence, non-invasive delivery systems for such drugs are required [3]. The oral route offers the advantage of self-administration with high patient compliance. However, the inherent poor permeability of the intestinal mucosa hampers oral, as well as other noninvasive administration routes [4]. To enable effective transmucosal protein delivery, attention has started to focus on formulations that improve intestinal absorption and prevent degradation [3,5]. Nano-sized systems (*e.g.*, liposomes, lipid and polymeric NPs, micelles) have been found to be advantageous over traditional formulations for protein delivery. Biotech drugs are predicted to become the main source of therapeutics in the near future [6]. Nevertheless, the therapeutic exploitation of such molecules will rely on the possibility to develop suitable formulations that can satisfactorily overcome the intrinsic limitations of their use; namely low oral bioavailability, and biological and physicochemical instability [7]. Indeed, the oral route is considered to be the most convenient and comfortable route of chronic drug administration for patients [8]. However, oral administration of protein drugs encounters many difficulties due to their proteolytic instabilities and limited abilities to traverse biological barriers [8]. Liposome and micelle-based delivery systems are not stable in the gut lumen and cannot elicit adequate protection to the sensitive biomacromolecules. Therefore, among the pharmaceutical formulations, NPs have been explored and found to be successful for drug delivery of peptidic drugs [9–11]. NPs, more specifically the biodegradable polymeric NPs, possess excellent biocompatibility, biodegradability, composition flexibility and small size, making them suitable for a variety of clinical applications. Furthermore, these formulations have been shown to enhance the absolute drug bioavailability following oral administration [12]. Although encapsulation with biodegradable polymers is very attractive, the manufacturing processes are still complicated and expensive for hydrophilic macromolecules. In the case of efficient entrapment approaches of protein drugs into these systems, encapsulation

Abbreviations: NPs, nanoparticles; SC, subcutaneous/ly; STMP, sodium trimetaphosphate; MPs, microparticles; Eudragit L, Eudragit® L100-55; HPMC, hydroxypropylmethylcellulose; GLP-1, glucagon-like peptide-1; BSA, bovine serum albumin; DDW, bi-distilled water; Cryo-TEM, cryogenic transmission electron microscopy; XHR SEM, extra high-resolution scanning electronic microscope; SD, Sprague–Dawley; AUC, area under the curve; PDI, poly dispersity index; PBS, phosphate buffered saline; CSK, CSKSSDYQC peptide.

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using polymers may provide (a) concealment and protection of the proteins from degradation during storage and delivery and (b) a controlled release profile when desired [13–15].

Consequently, the development of sophisticated, nanotechnological delivery systems for oral administration of protein drugs, including exenatide, presents an alluring scientific and pharmaceutical challenge. Recently, some authors have reported that they were able to improve the oral bioavailability of novel glucagon-like peptide-1 (GLP-1) analogs, relative to their subcutaneous (SC) counterpart, by 14.0 ± 1.8% [16,17]. Although this achievement is promising, it is much less than the minimal oral absolute bioavailability of 25–30%, which is still considered poor by the FDA for an orally administered drug.

Exenatide, a 39-amino-acid peptide, is a synthetic version of exendin-4, a hormone found in lizard saliva that displays biological properties similar to human GLP-1, a regulator of glucose metabolism and insulin secretion (insulinotropic) [18,19]. The medication is injected SC twice a day (b.i.d.) using a filled pen device, stimulates insulin secretion, lowers blood glucose in mammals, and has been found to be effective in the diabetic state [20].

The medicine is available in two dose strengths: 5 μg and 10 μg. Treatment often begins with the 5 μg dosage, which is increased if adverse effects are insignificant. It should be emphasized that a potential disadvantage of exenatide is the frequent SC injections required. SC injections can cause pain, side effects and possible infections at the sites of injection that could adversely affect patient compliance [10,20]. A long-acting release form of exenatide has been developed for use as a once-weekly injection. This sustained-release formulation consists of injectable microspheres of exenatide and poly (D,L lactic-co-glycolic acid), a common biodegradable polymer with established use in absorbable sutures and extended-release pharmaceuticals, which allow gradual drug delivery at a controlled rate [21,22]. It should be mentioned that the short-acting preparation of exenatide offers the additional benefit of greater decelerating gastric emptying, which appears to be the key factor driving the reduction of postprandial glycemia [23]. Thus, exenatide extended release is a useful option for the treatment of type 2 diabetes, particularly in patients where body weight loss is an essential aspect of the individual patient's management. However, it is still an injection.

There is no doubt that the oral route remains the preferred route of drug administration and efforts should be invested to develop an oral delivery system of exenatide ensuring appropriate therapeutic activity and optimum patience compliance.

The objective of the present study is to design and evaluate an effective combined nano-in-micro encapsulation delivery system which will markedly enhance the oral bioavailability of exenatide.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA, glutaraldehyde 8% (in water) and dextran 12 kDa were purchased from Sigma-Aldrich (Rehovot, Israel). Exenatide was kindly donated by Teva Pharmaceuticals (Jerusalem, Israel). STMP was purchased from Alfa Aesar (Ward Hill, MA, USA). Poly(methacrylic acid, Ethyl acrylate) 1:1 (Eudragit® L100-55) was obtained from Rohm (Dramstadt GmbH, Germany). HPMC (Methocel E4M Premium) was purchased from Dow Chemical Company (Midland, MI, USA). Sodium phosphate monobasic, monohydrate was purchased from Mallinckrodt Chemicals (Phillipsburg, NJ, USA). Phosphate buffered saline was obtained from Biological Industries (Kibbutz Beit Haemek, Israel). All organic solvents were HPLC grade and purchased from J.T. Baker (Deventer, Holland).

2.2. Preparation of primary NPs

The first line of protection on the sensitive bio-macromolecule, exenatide, was achieved by loading the peptide into primary BSA NPs. Two different types of NPs were prepared: BSA NPs cross-linked with glutaraldehyde 8% and BSA combined with dextran 12 kDa NPs cross-linked with STMP.

2.3. BSA NPs cross-linked with glutaraldehyde

The BSA NPs cross-linked with glutaraldehyde were prepared using an established desolvation method as previously described by Weber et al. [24]. 200 mg of BSA and 4 or 8 mg of exenatide were dissolved in 20 mL of bi-distilled water (DDW). After 0.5 h, the pH of the solution was adjusted to 8.5 by 0.1 N NaOH solution. Then, 40 mL of acetone was slowly added to the aqueous phase. A dispersion was formed as evidenced by the rapid formation of opalescence in the dispersion medium. BSA NPs were then cross-linked using 12.5 μL of glutaraldehyde 8% solution for 2 h. Following completion of the cross-linking reaction, the acetone was evaporated under laminar air flow.

2.4. BSA/dextran NPs cross-linked with STMP

The BSA/dextran NPs were similarly prepared by dissolving in 20 mL DDW, the following compounds: 200 mg of BSA, 50 mg of dextran 12 kDa and 4 or 8 mg of exenatide. After 0.5 h, the pH of the solution was adjusted to 8.5 by 0.1 N NaOH solution to ensure that the adjacent hydroxyl groups on dextran were available for the reaction with the STMP cross-linker. Then, 20 mL of acetone were slowly added to the aqueous phase. BSA/dextran NPs were then cross-linked using 1 mL of 5% STMP solution (in DDW) for 3 h and acetone was evaporated as described above. Preliminary formulations were prepared and evaluated by varying the process parameters. Two formulations that differed in the dextran amount were selected for further animal studies: 50 and 150 mg.

2.5. Microparticles (MPs) preparation

The MPs were formed by microencapsulating the exenatide-loaded NPs using the spray-drying technique. For the purpose of microencapsulation, 100 mL of NaH_2PO_4 buffer was prepared. The pH of the buffer was adjusted to 6.5 with 1 N NaOH solution. An amount of 750 mg of Eudragit L was dissolved in this solution maintaining pH at 6.5. In addition, 1% w/v HPMC solution was prepared by adding 1000 mg of HPMC to 100 mL of pre-heated (~80 °C) DDW. The Eudragit L solution was then added *via* a funnel through a gauze (to filter any undissolved Eudragit L particles) to the HPMC solution. After ensuring that the entire acetone had evaporated from the NPs suspension, the combined solution of the MP polymers was added to the NPs suspension. The suspension was then spray-dried with a Buchi mini spray-drier B-290 apparatus (Flawil, Switzerland) under the following conditions: inlet temperature 160 °C; outlet temperature 85 °C; aspiration 100%; feeding rate of the suspension was 3.33 mL/min; the powder was collected in the cyclone separator and the outlet yield was calculated.

In order to achieve the optimal *in vitro* release profile, several formulations with different ratios of HPMC:Eudragit L were prepared and evaluated as follows: 0.5:0.75, 0.75:0.5, 0.75:0.75, 0.75:1, 1:0.5 and 1:0.75 respectively (all values are at %w/v).

2.6. Physicochemical characterization of the NPs and subsequent MPs

2.6.1. NPs characterization

The mean diameter and zeta potential of the various NPs were characterized using Malvern's Zetasizer (Nano series, Nanos-ZS, UK) at 25 °C using water as a diluent. Morphological evaluation was performed using cryogenic transmission electron microscopy (Cryo-TEM). In the Cryo-TEM method, a drop of the solution is placed on a carbon-coated perforated polymer film supported on a 300-mesh Cu grid (Ted Pella Ltd., Redding, CA, USA), and the specimen is automatically vitrified using Vitrobot (FEI) by means of a fast quench in liquid ethane to −170 °C.