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Thiolated Eudragit nanoparticles for oral insulin delivery: Preparation, characterization and in vivo evaluation

Yan Zhang[a], Xiaorong Wu[a], Lingkuo Meng[a], Yu Zhang[a], Ruiting Ai[b], Na Qi[a], Haibing He[a], Hui Xu[a], Xing Tang[a,*]

[a] Department of Pharmaceutics, Shenyang Pharmaceutical University, Shenyang, 110016, China
[b] National Center for Biotechnology Development, Beijing, China

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ABSTRACT

In the present study thiolated Eudragit L100 (Eul) based polymeric nanoparticles (NPs) were employed to develop an oral insulin delivery system. Sulfydryl modification was achieved by grafting cysteine to the carboxylic acid group of Eudragit L100, which displayed maximum conjugate level of 390.3 ± 13.4 μmol thiol groups per gram. Eudragit L100-cysteine (Eul-cys) and Eul nanoparticles were prepared by the precipitation method, in which reversible swelling of pH-sensitive material was used for insulin loading and release. Nanoparticles were characterized in terms of their particle size, morphology, loading efficiency (LE%) and in vitro insulin release behavior. The NPs had an average size of 324.2 ± 39.0 nm and 308.8 ± 35.7 nm, maximal LE% of 92.2 ± 1.7% and 96.4 ± 0.5% for Eul-cys and Eul, respectively. The release profile of NPs in vitro showed pH-dependent behavior. Circular dichroism (CD) spectroscopy analysis proved that the secondary structure of the insulin released from NPs was unchanged compared with native insulin. The mucoadhesion study in vitro showed that Eul-cys NPs produced a 3-fold and 2.8-fold increase in rat jejunum and ileum compared with unmodified polymer NPs, respectively, which was due to the immobilization of thiol groups on Eudragit L100. Oral administration of insulin-loaded Eul-cys NPs produced a higher and prolonged hypoglycemic action, and the corresponding relative bioavailability of insulin was found to be 7.33 ± 0.33%, an increase of 2.8-fold compared with Eul NPs (2.65 ± 0.63%). This delivery system is a promising novel tool to improve the absorption of protein and peptide drugs in the intestinal tract.

1. Introduction

Insulin, a peptide drug, is still indispensable for patients with insulin-dependent diabetes mellitus (IDDM). Subcutaneous injection of insulin is currently the standard treatment for diabetic patients. However, multiple daily injections are the major disadvantage and cause patients a great deal of pain (Chin et al., 1993; Lassmann-Vague and Raccah, 2006). Oral administration of insulin can be beneficial to diabetic patients as it could not only reduce the pain resulting in good compliance, but also mimic the physiological fate of insulin (Sonaje et al., 2010). Therefore, it is recognized as the natural and ideal route for drug administration. However, the oral absorption of insulin is severely limited because of its inactivation by proteolytic enzymes in the gastrointestinal tract and its low permeability through the intestinal membranes (Carino and Mathiowitz, 1999).

Over recent decades, various strategies have been proposed to improve the stability and absorption of insulin within the GIT, including the use of permeation enhancers (Maroni et al., 2012; Zhao et al., 2011), protease inhibitors (Marschütz and Bernkop-Schnürch, 2000; Su et al., 2012), microsphere and nanoparticulate encapsulation (Woitiski et al., 2009; Zhang et al., 2011), hydrogels (Morishita et al., 2006), liposomes (Kisel et al., 2001), and mucoadhesion formulations (Whitehead et al., 2004). Their single and co-administration have shown some advantages and boosted oral insulin bioavailability. Nevertheless, some of these approaches also produced several negative effects such as irritation of the intestinal mucosal membrane and irreversible damage to the membrane barrier (Carino and Mathiowitz, 1999). Polymeric nanoparticles with biodegradable and biocompatible polymers are good candidates for particulate carriers to deliver peptide drugs. Polymers used for the formulation of nanoparticles, such as chitosans, poly(acrylic acid)

Abbreviations: Eul, Eudragit L100; Cys, L-cysteine hydrochloride; Eul-cys, Eudragit L100-cysteine; NPs, nanoparticles; LE%, loading efficiency; LC%, loading capacity; PA, pharmacological bioavailability; CD, circular dichroism; GIT, gastrointestinal tract; PI, isoelectric point; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; NHS, N-hydroxysuccinimide; BGL, blood glucose level.

* Corresponding author at: No. 103, Wenhua Road, Shenyang, Zip code 110016, China. Tel.: +86 024 23986343; fax: +86 024 23911736.
E-mail address: tangpharm@yahoo.com.cn (X. Tang).

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and their derivatives, have been widely reported, and nanoencapsulation of peptides and protein colloidal particles can protect them against the harsh environment of the gastrointestinal tract and enhance their transmucosal transport (Lehr et al., 1992; van der Lubben et al., 2001). Moreover, they can prolong the residence time of the drug at the site of absorption and minimize drug dilution and degradation by the luminal contents owing to their mucoadhesion, which is also recognized to improve the bioavailability of oral insulin delivery (Makhlof et al., 2011).

Recently, pH-sensitive polymers of Eudragit® Series were widely applied in the delivery system of protein drug (Damgé et al., 2010; Jelvehgari et al., 2010; Mundargi et al., 2011). The traditional enteric coating-type materials have shown the potential to be the vehicles of the macromolecule, such as insulin. However, the hypoglycemic effect after administration of Eudragit insulin microspheres alone was unsatisfactory (Morishita et al., 1993). In the present study, Eudragit L100 was chosen as the matrix polymer and the sulfhydryl groups were brought onto its backbone. Thiolated polymers have been developed for their strong mucoadhesive properties and were verified to be effective for the transmucosal delivery of proteins and polypeptides (Bernkop-Schnurch, 2005). So we attempted to develop thiolated Eudragit nanoparticles for insulin mucosal application. Improved mucoadhesive properties are guaranteed on one hand and the distribution of multiparticulate systems is more uniform compared with single-unit preparations on the other hand (Bernkop-Schnürch et al., 2006).

The aim of this study was to prepare and characterize NPs based on thiolated Eudragit L100 and unmodified polymer and evaluate their potential for the transportation of insulin in rats. The formulations of insulin-loaded NPs made of Eul-cys and Eul were designed and characterized including the particle size distribution, morphology, insulin loading and in vitro release, mucoadhesion in different rat intestinal segments. The secondary structure of insulin was investigated by CD spectroscopy and the biological efficacy after oral administration of NPs in streptozotocin-induced diabetic rats was studied.

2. Materials and methods

2.1. Materials

Porcine insulin (27.8 IU/mg) was purchased from Xuzhou Wanbang Biochemical Pharmaceutical Co., Ltd., China. Eudragit® L-100 was obtained from Ionic Degussa (China). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), 5,5-dithiobis (2-nitrobenzoic acid) (Ellman's reagent), L-cysteine hydrochloride (cys) and 6-amino-fluorescein were purchased from Sigma (St. Louis, MO, USA). D-trehalose was purchased from Zhongnuo Biological Engineering Co., Ltd. (Nanning, China). Enteric-coated capsules for rats were obtained from Qiangji Pharmaceutical Capsule Factory (Chaozhou, China). All other reagents were of analytic grade.

Wistar rats (250 ± 30 g) were purchased from the Experimental Animal Center of Shenyang Pharmaceutical University and treated according to protocols evaluated and approved by the University Ethical Committee during the entire study.

2.2. Preparation of polymer–cysteine conjugates

The covalent attachment of cysteine hydrochloride to Eudragit was achieved by the formation of amide bonds between the primary amino group of the sulfydryl compound and a carboxylic acid group of the polymer (Fig. 1) and the procedure was adopted from Bernkop-Schnürch et al. (Bernkop-Schnürch et al., 2001), with minor modifications. Briefly, 0.5 g Eudragit L100 was hydrated in 50 mL demineralized water, which was neutralized by 10% NaOH

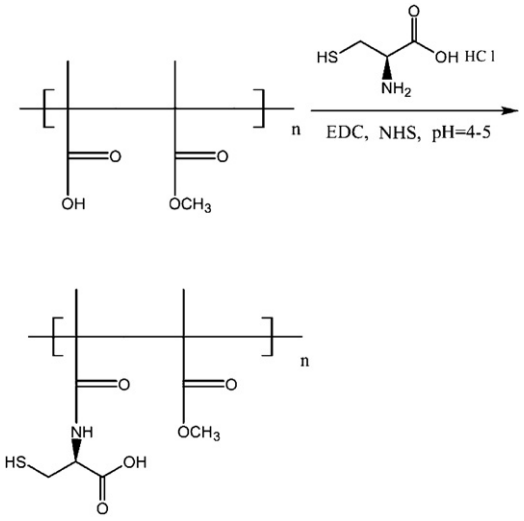

Fig. 1. Synthetic route of Eul-cys conjugates.

(w/v). The required amounts of NaOH were calculated after titration based on the European Pharmacopoeia Method (2010). The carboxylic acid moieties of the polymers were activated for 30 min by the addition of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-Hydroxysuccinimide (NHS). Cysteine hydrochloride was added and the pH was adjusted within the range of 4–5. The amounts of the different reagents are listed in Table 1. Reaction mixtures were incubated for 3 h under permanent stirring at room temperature. The resulting polymer–cysteine conjugates were isolated in the dark by dialyzing at 10 °C against 1 mM HCl, then twice against the same medium but containing 1% NaCl and then exhaustively against 1 mM HCl. Control samples were prepared and isolated in exactly the same way as the polymer–cysteine conjugates but EDC and NHS were omitted during the coupling reaction. All samples were lyophilized at −30 °C and 0.01 mbar. Polymer–cysteine conjugates and controls were stored at 4 °C until required for further investigation.

2.3. Characterization of polymer conjugates

The amount of thiol groups immobilized on the polymer was determined with Ellman's reagent according to a method described previously (Bernkop-Schnürch et al., 1999). First, 5 mg samples of both the conjugates and control were hydrated in 2.5 mL of deionized water, aliquots of 250 μL were withdrawn, and mixed with 250 μL 0.5 M phosphate buffer, pH 8.0 and 500 μL Ellman's reagent (3 mg 5,5′-dithiobis(2-nitrobenzoic acid) dissolved in 10 mL of 0.5 M phosphate buffer pH 8.0). The samples were incubated for 2 h at room temperature, protected from light. Then, 300 μL of each sample was transferred to a microplate and the absorbance

Table 1
Amount of L-cysteine covalently attached to Eudragit L100 and amount of EDC used for synthesis of each thiomer.

No.	mass of EudragitL 100 (g)	Added L-cysteine HCl (g)	EDC (final conc., mM)	NHS (mM)
1	0.5	2	50	–
2	0.5	1	50	–
3	0.5	0.5	50	–
4	0.5	0.5	100	–
5	0.5	0.5	200	–
6	0.5	2	100	100
7	0.5	1	100	100
8	0.5	1	–	–