European Journal of Pharmaceutics and Biopharmaceutics 80 (2012) 95–102

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Thiolated chitosan: Development and in vivo evaluation of an oral delivery system for leuprolide

Javed Iqbal, Gul Shahnaz, Glen Perera, Fabian Hintzen, Federica Sarti, Andreas Bernkop-Schnürch *

Department of Pharmaceutical Technology, Institute of Pharmacy, University of Innsbruck, Josef Möller Haus, Innsbruck, Austria

A R T I C L E I N F O

Article history:
Received 29 November 2010
Accepted in revised form 15 September 2011
Available online 22 September 2011

Keywords:
Thiomers
Chitosan–TGA
Leuprolide
Tight junctions
Oral drug delivery

A B S T R A C T

The aim of the present study was to develop an oral delivery system for the peptide drug leuprolide. Gel formulations based on unmodified chitosan/reduced glutathione (GSH) and chitosan–thioglycolic acid (chitosan–TGA)/GSH were prepared, and their effect on the absorption of leuprolide was evaluated in vitro and in vivo in male Sprague Dawley rats. Transport studies were performed with freshly excised rat intestinal mucosa mounted in Ussing-type chambers. Due to the addition of gel formulations comprising 0.5% (m/v) unmodified chitosan/0.5% (m/v) GSH and 0.5% (m/v) chitosan–TGA/0.5% (m/v) GSH, the transport of leuprolide across excised mucosa was improved up to 2.06-fold and 3.79-fold, respectively, in comparison with leuprolide applied in buffer ($P_{app} = 2.87 \pm 0.77 \times 10^{-6}$ cm/s).

In vivo, the addition of oral gel formulation comprising 8 mg of unmodified chitosan, 1 mg of GSH and 1 mg of leuprolide increased the area under the plasma concentration–time curve (AUC_{0-8}) of leuprolide 1.39-fold in comparison with leuprolide having been administered just in saline. Moreover, the administration of oral gel formulation comprising 8 mg of chitosan–TGA, 1 mg of GSH and 1 mg of leuprolide resulted in a further enhanced leuprolide plasma concentration, and the area under the plasma concentration–time curve (AUC_{0-8}) of leuprolide was increased 3.72-fold in comparison with the control. With the oral gel formulation comprising 8 mg of chitosan–TGA, a relative bioavailability (versus s.c. injection) of 4.5% was achieved in contrast to the control displaying a relative bioavailability of 1.2%. Thus, according to the achieved results, it is suggested that chitosan–TGA in combination with GSH is a valuable tool for improving the oral bioavailability of the peptide drug leuprolide.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Administration of therapeutic peptide and protein drugs still remains a challenge to the pharmaceutical industry. Due to their poor oral bioavailability most protein and peptide drugs are currently administered via parenteral routes, which is often painful and inconvenient. Thus, development of non-invasive delivery systems for these hydrophilic macromolecules is strongly on demand.

Leuprolide acetate is a synthetic nanopeptide and a potent agonist of the luteinizing hormone-releasing hormone (GnRH or LH–RH) receptor. It is one of the most widely used agents for the non-surgical hormonal treatment of advanced prostate cancer and also found to be effective in the treatment of non-cancer indications and hormone-dependent diseases such as endometriosis, uterine fibroids and central precocious puberty in children [1,2]. Like most other peptide and protein drugs, leuprolide has a poor permeability across intestinal epithelia which results in a very low oral bioavailability of around 1–2% depending on the animal species. Rapid degradation of leuprolide by chymotrypsin and rat intestinal mucosa homogenates has already been reported [3,4]. Due to low oral bioavailability, leuprolide is currently administered via subcutaneous, intramuscular and intranasal routes [5]. Undoubtedly, oral route is the most convenient and efforts are under way to identify and rectify the absorption problems associated with the GI-tract. Ping et al., for instance, investigated the transport properties, mechanisms and causes of low bioavailability of leuprolide and demonstrated that the peptide followed the passive diffusion (paracellular) pathway, and transport of leuprolide was improved by the addition of chitosan, EDTA and trypsin inhibitor [6]. These results suggest that the opening of tight junctions and the use of enzyme inhibitory agents would be the key factors to improve the intestinal absorption of leuprolide.

Over the past few years, thiolated polymers or so-called thiomers have appeared as a promising excipient for the delivery of drugs including peptides [7–9]. Due to the formation of inter- and intramolecular disulphide bonds within thiomers or with mucus glycoproteins, they display mucoadhesive properties, prolonged disintegration time and a comparatively more controlled release of incorporated drugs. Furthermore, thiolated

* Corresponding author. Institute of Pharmacy, Leopold-Franzens-University Innsbruck, Josef-Möller-Haus, Innrain 52c, A-6020 Innsbruck, Austria. Tel.: +43 512 507 5383; fax: +43 512 507 2933.

E-mail address: andreas.bernkop@uibk.ac.at (A. Bernkop-Schnürch).

0939-6411/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2011.09.010

polymers particularly in combination with reduced glutathione (GSH) were shown to improve the uptake of hydrophilic macromolecules from the GI-tract [10]. The mechanism responsible for the permeation-enhancing effect of thiomers seems to be based on the inhibition of protein tyrosine phosphatase, being involved in the closing process of tight junctions, via a GSH-mediated mechanism [11]. Because of their high molecular mass, thiomers are not absorbed from the GI-tract and remain therefore located in the GI-tract at the site of drug absorption. Thus, systemic side effects can be excluded by the use of these thiolated polymeric excipients as drug carriers. To the best of our knowledge, to date, no data exist about the gel formulations based on chitosan derivatives (chitosan–TGA) for oral peptide delivery system. The use of mucoadhesive oral gel was thought to offer advantage of prolonged residence time of the dosage form at the site of absorption. Therefore, an enhanced oral bioavailability could be achieved resulting in many cases in a reduced dosing frequency and patient compliance. Consequently, thiolated chitosan/GSH gel formulation could be a valuable tool for oral delivery of various therapeutic peptides. It was therefore the aim of the present study to evaluate the effect of thiolated chitosan/GSH gel formulation for oral delivery of the poorly permeable peptide drug leuprolide.

2. Materials and methods

2.1. Materials

Leuprolide (H-4060) and internal standard (IS) (H-4070) were purchased from Bachem AG, Switzerland. Chitosan highly viscous (⩾400 Pa s, 500–700 kDa, degree of deacetylation 75–85%) was obtained from Fluka, Buchs, Switzerland. Sodium borohydride, L-glutathione reduced form (GSH), L-cysteine hydrochloride (anhydrous, minimum 98%), Ellman's reagent (DTNB, 5,5′-dithiobis (2-nitrobenzoic acid)), thioglycolic acid (TGA), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC) and *N*-(2-hxdroxyethyl)-piperazine-*N*'-(2-ethanesulfonic acid) (HEPES) were obtained from Sigma-Aldrich, St. Louis, MO.

2.2. Synthesis of the chitosan–TGA

First, 500 mg of chitosan was hydrated in 4 mL of 1 M HCl and dissolved by the addition of demineralised water to obtain a 1% solution of chitosan hydrochloride. Thereafter, 500 mg of TGA was added. After TGA was completely dissolved in the chitosan hydrochloride solution, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC) was added in a final concentration of 125 mM in order to activate the carboxylic acid moieties of TGA. The reaction mixture was incubated at pH 5 for 3 h at room temperature under stirring. Sample prepared in exactly the same way but omitting EDAC during the coupling reaction served as a control for the analytical studies [12].

In order to eliminate unreacted TGA and to isolate the polymer conjugates, the reaction mixtures were dialysed five times in tubings (molecular weight cut-off 12 kDa; dialysis tubings, cellulose membrane; Sigma, St. Louis, MO) for 3 days in total at 10 °C in the dark. In detail, the polymer conjugates were dialysed one time against 5 mM HCl, then two times against the same medium but containing 1% NaCl to quench ionic interactions between the cationic polymer and the anionic sulfhydryl compound. Finally, the polymer conjugates were dialysed exhaustively two times against 1 mM HCl to adjust the pH of the polymer solutions to 4. Thereafter, chitosan–TGA conjugate and control were lyophilised by drying frozen aqueous polymer solutions at −70 °C and 0.01 mbar (VirTis benchtop 6 K freeze dryer; NY, USA) and stored at 4 °C until further use.

2.3. Determination of thiol group content

The amount of thiol groups immobilized on chitosan was spectrophotometrically determined with Ellman's reagent. First, 0.5 mg of each of the modified and unmodified polymer conjugates was hydrated in 250 μL of demineralised water separately. Then, 250 μL of 0.5 M phosphate buffer (pH 8.0) and 500 μL of Ellman's reagent (3 mg in 10 mL of 0.5 M phosphate buffer pH 8.0) were added. The samples were incubated for 3 h at room temperature. The supernatant was separated from the precipitated polymers by centrifugation (24,000*g*, 5 min). Thereafter, 300 μL of the supernatant was transferred to a microtitration plate, and the absorbance was measured at a wavelength of 450/620 nm with a microtitration-plate reader (Tecan infinite M200 spectrophotometer, Grödig, Austria). TGA standards were used to calculate the amount of thiol groups immobilised on the polymer [7].

2.4. Formation of disulphide bonds

Disulphide content was measured after the reduction with $NaBH_4$ and addition of 5,5′-dithiobis(2-nitrobenzoic acid). To determine the total amount of bound thiol functions, 0.5 mg of the polymer conjugates was hydrated in 350 μL of demineralized water and then 650 μL of 0.05 M phosphate buffer (pH 6.8) was added. After a swelling process of 30 min, 1 mL of a freshly prepared 4% (m/v) solution of sodium borohydride was added to the polymer suspensions. The mixtures were incubated for 1 h in an oscillating water bath at 37 ± 0.5 °C. Thereafter, 200 μL of 5 M HCl was added, and the reaction mixtures were agitated for 10 min in order to destroy the remaining sodium-borohydride. The solutions were neutralised by the addition of 1 mL 1 M phosphate buffer (pH 8.5) and 100 μL of 0.4% (m/v) Ellman's reagent dissolved in 0.5 M phosphate buffer (pH 8.0) were immediately added. After incubation for 1 h at room temperature, aliquots of 250 μL were transferred to a microtitration plate and the absorbance was measured at 450 nm with a microtitration-plate reader (Tecan infinite M200 spectrophotometer, Grödig, Austria). The quantity of bound TGA was calculated using a standard curve obtained by the thiol group determination of a series of solutions containing increasing concentrations of L-cysteine hydrochloride. The amount of disulphide bonds was calculated by subtracting the quantity of free thiol groups from the total thiol moieties present on the polymer conjugates [7].

2.5. Tensile studies

The adhesive strength of gel formulations based on (0.5% m/v) chitosan–TGA and (0.5% m/v) unmodified chitosan was evaluated using excised porcine intestinal mucosa by applying some modifications in a previously described method [13]. Briefly, porcine mucosa was glued to a glass slide (thickness 1.0 mm, dimensions of 26 × 76 mm) using a cyanoacrylate adhesive and hung from a laboratory stand with a nylon thread (15 cm). Then 10 mg of the gel was uniformly spread over another segment of porcine mucosa, already glued to a glass platform and placed on a balance. Mobile platform was then carefully raised until the gel layer came in contact with the hanging mucosal segment. The contact was determined when the nylon thread holding the glass slide became slightly bent. After a contact time of 20 min, the mobile platform was pulled down from upper mucosal segment at a rate of 0.1 mm/s. Data points were collected every second by computer software (SartoCollect V 1.0; Satorius AG, Germany) linked to the balance with integrated interface. Data were transferred to EXCEL 2007 (Microsoft, USA), and the force versus displacement curves were analysed to calculate the maximum force of detachment

Download English Version:

https://daneshyari.com/en/article/2085367

Download Persian Version:

https://daneshyari.com/article/2085367

Daneshyari.com