Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmaceutics and Biopharmaceutics 68 (2008) 260–266
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Note

Novel preparation of enteric-coated chitosan-prednisolone conjugate microspheres and in vitro evaluation of their potential as a colonic delivery system

Tomoko Oosegi, Hiraku Onishi *, Yoshiharu Machida

Department of Drug Delivery Research, Hoshi University, Tokyo, Japan

Received 22 December 2006; accepted in revised form 22 June 2007
Available online 4 July 2007

Abstract

After chitosan-succinyl-prednisolone conjugate (Ch-SP) was synthesized, conjugate microspheres (Ch-SP-MS), Eudragit L100-coated Ch-SP-MS and Eudragit S100-coated Ch-SP-MS, were prepared under novel preparative conditions. Namely, sonication was utilized to prepare finer Ch-SP-MS, and the addition ratio of Eudragit was reduced to yield Eudragit-coated Ch-SP-MS with higher drug content. Ch-SP-MS and Eudragit-coated Ch-SP-MS had mean sizes of 1.3 μm and approximately 30 μm, respectively, and showed prednisolone (PD) contents of 4.6% (w/w) and approximately 3% (w/w), respectively. Morphological changes of all the types of microparticles in different pH media were observed by scanning electron microscopy and confocal laser scanning microscopy. Both methods gave similar results. Both types of Eudragit-coated Ch-SP-MS protected Ch-SP-MS from morphological change at pH 1.2, and regenerated Ch-SP-MS fast at pH 6.8 and 7.4. For all types of microparticles, release of PD was suppressed at pH 1.2, but caused gradually at pH 6.8. These particle characteristics and in vitro behaviors demonstrated that the present Eudragit-coated Ch-SP-MS were considered potentially suitable for in vivo or practical application as a specific delivery system of PD to IBD sites.

Keywords: Chitosan-prednisolone conjugate microspheres; Eudragit-coated microparticles; Particle characteristics; Morphological change; Drug release; Specific delivery

1. Introduction

In the treatment of inflammatory bowel disease (IBD), 5-aminosalicylic acid (5-ASA) and steroidal or non-steroidal anti-inflammatory drugs are frequently administered orally to the patients [1]. Administration of these drugs at a large and frequent dose for a long period causes significant and prolonged absorption of the drugs from the small intestine, often leading to toxic side effects [2]. Therefore, the specific delivery of drugs to diseased parts has been developed. For example, salazosulfapyridine, a prodrug of 5-ASA, [3] and Pentasa, acting as a delayed release system of 5-ASA, [4] are clinically available; however, they are not necessarily satisfactory, and more improved systems are expected.

Recently, a chitosan capsule containing 5-ASA was demonstrated to display an excellent effect against 2, 4, 6-trinitrobenzene sulfonic acid (TNBS)-induced colitis in rats [5]. Also, micro- or nano-particulate dosage forms have been found to be effective to deliver drugs to the intestine, Peyer's patches or colon [6–9]. Small particles can penetrate the mucus layer more deeply, and reach the diseased sites well [7]. In these systems, biocompatible and biodegradable polymers are often used [5,8,9], and their degradation by enzymes of bacteria and macrophages in the diseased region can accelerate drug release.

Chitosan is a biocompatible and biodegradable polymer, and is considered to be useful as a material for oral drug delivery systems due to its safety [10]. In order to

* Corresponding author. Department of Drug Delivery Research, Hoshi University, 2-4-41, Ebara, Shinagawa-ku, Tokyo 142-8501, Japan. Tel.: +81 3 5498 5724; fax: +81 3 3787 0036.
E-mail address: onishi@hoshi.ac.jp (H. Onishi).

0939-6411/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2007.06.016

produce microparticulate system with the ability to show the gradual drug release and efficient delivery, we synthesized a chitosan-succinyl-prednisolone conjugate (Ch-SP) as a macromolecular prodrug of PD, and prepared the microspheres (Ch-SP-MS) using Ch-SP [11,12]. Although Eudragit coating of Ch-SP-MS was attempted previously [12], the size of Ch-SP-MS and the drug content of Eudragit-coated Ch-SP-MS were not necessarily satisfactory. In this study, novel preparation conditions were examined to obtain refined Ch-SP-MS and their Eudragit L100- or S100-coated Ch-SP-MS. All the microparticles were evaluated based on particle size, morphology and drug release.

2. Materials and methods

2.1. Materials

Prednisolone (PD) and prednisolone 21-hemisuccinate (SP) sodium salt (SP-Na) were obtained from Sigma–Aldrich Co. (St. Louis, MO, USA). Chitosan (Ch) (viscosity grade = 1000 (5 g/l, 20 °C), deacetylation degree = 80% (mol/mol)), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and fluorescein isothiocyanate (FITC) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Eudragit L100 and Eudragit S100 were obtained from Rohm GmbH Chemische Fabrik (Darmstadt, Germany). Sorbitan sesquioleate (SO-15) was purchased from Nikko Chemicals Co., Ltd. (Tokyo, Japan). All other chemicals were of reagent grade.

2.2. Preparation of chitosan microspheres

Ch-SP conjugate microspheres (Ch-SP-MS) and FITC-labeled chitosan microspheres (FITC-Ch-MS) were prepared. Ch-SP conjugate (Ch-SP) was prepared as follows [11,12]. Ch (120 mg) was dissolved in water by adjusting the solvent pH to 3 with a 1 M HCl aqueous solution. SP-Na (40 mg) was dissolved in 5 ml of water, and added to the Ch solution. The pH of the mixture was adjusted to pH 5.5 with a 1 M NaOH aqueous solution. EDCI (200 mg) dissolved in 5 ml of water was added to the solution containing Ch and SP. The reaction mixture was stirred at 900 rpm under ice cooling for the first 5 h and at room temperature for 19 h. Further, 5 ml of water containing 200 mg EDCI was added, and the mixture was stirred at 900 rpm at room temperature for another 24 h. The reaction mixture was added to a fourfold volume of acetone to precipitate Ch-SP. Ch-SP was washed with a mixture of acetone and water (4:1, v/v), suspended in 60 ml of water, and lyophilized to obtain the Ch-SP powder.

FITC-labeled chitosan (FITC-Ch) was prepared in the following manner. Ch (500 mg) was dissolved in water by adjusting the pH to 3 with a 1 M HCl aqueous solution. After the pH was adjusted to 6.5 with a 1 M NaOH aqueous solution, FITC (15 mg) was added. The resultant mixture was stirred for 24 h in the dark, and the pH was adjusted to 9 with a 1 M NaOH aqueous solution. The precipitate was collected after centrifugation at 3000 rpm for 5 min, washed by repeating dissolution at pH 3 (HCl aqueous solution) and subsequent precipitation at pH 9 (NaOH aqueous solution), dispersed in water, and lyophilized to obtain FITC-Ch powder.

Ch-SP-MS were prepared by emulsification and subsequent evaporation using Ch-SP. Ch-SP (50 mg) was dissolved in a 1% (v/v) acetic acid aqueous solution, and emulsified in 150 ml of liquid paraffin containing 1% (w/v) SO-15 at 1200 rpm at 70 °C. Stirring was continued at 70 °C for 20 min, and at 80 °C for 10 min. Then, the emulsion was sonicated at 80 °C under 28 kHz (100 W) for 10 min using an ultrasonicator VS-100III SUNPAR (IUCHISEIEIDO, Japan). The resultant mixture was stirred at 400 rpm at 100 °C for 1 h. After the mixture was cooled to room temperature, the same volume of *n*-hexane was added, and the mixture was centrifuged at 3000 rpm for 5 min to precipitate the product. The product was washed with *n*-hexane, and dried in a desiccator to obtain Ch-SP-MS. FITC-Ch-MS were prepared in the same manner as Ch-SP-MS, except that FITC-Ch was used instead of Ch-SP.

2.3. Eudragit coating of chitosan microspheres

Eudragit L100 or S100 (130 mg) was dissolved in 1 ml of methanol, and Ch-SP-MS (130 mg) was added. The methanol suspension was dropped in 50 ml of liquid paraffin containing 2% (w/v) SO-15, which was stirred at 600 rpm at 40 °C. Stirring at 40 °C was continued under reduced pressure to evaporate methanol completely. Then, the mixture was cooled to room temperature, and the same volume of *n*-hexane was added, and the resultant mixture was centrifuged at 3000 rpm for 5 min to precipitate the product. The product was washed with *n*-hexane, and dried in a desiccator to obtain Eudragit L100-coated Ch-SP-MS (Ch-SP-MS/EuL) or Eudragit S100-coated Ch-SP-MS (Ch-SP-MS/EuS). Eudragit L100-coated FITC-Ch-MS (FITC-Ch-MS/EuL) or Eudragit S100-coated FITC-Ch-MS (FITC-Ch-MS/EuS) were prepared in the same manner except that FITC-Ch-MS were used instead of Ch-SP-MS.

2.4. High performance liquid chromatography (HPLC)

HPLC assay was used for the determination of PD in the sample solution. The HPLC system consisted of an LC-10AS pump, an SPD-10A spectrophotometric detector, a C-R7 chromatopac, an SCL-10A system controller, an SIL-10A autosampler and a CTO-10A column oven (Shimadzu Corp., Japan). The detector was set at 246 nm, and the column oven was set at 30 °C. A Spelcosil LC-18-DB column (4.6 mm in inner diameter × 150 mm in length, particle size 3 μm; SUPELCO, USA) was used as an analytical column. A 22% (w/v) 2-propanol aqueous solution containing 0.1% (w/v) trifluoroacetic acid was used as the mobile phase, and the flow rate was 1.0 ml/min. The absolute calibration curve method was applied to quantification analysis.