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Mucoadhesive microparticulates based on polysaccharide for target dual drug delivery of 5-aminosalicylic acid and curcumin to inflamed colon

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ABSTRACT

In this work, thiolated chitosan/alginate composite microparticulates (CMPs) coated by Eudragit S-100 were developed for colon-specific delivery of 5-aminosalicylic acid (5-ASA) and curcumin (CUR), and the use of it as a multi drug delivery system for the treatment of colitis. The physicochemical properties of the CMPs were evaluated. *In vitro* release was performed in gradually pH-changing medium simulating the conditions of different parts of GIT, and the results showed that the Eudragit S-100 coating has a pH-sensitive release property, which can avoid drug being released at a pH lower than 7. An everted sac method was used to evaluate the mucoadhesion of CMPs. *Ex vivo* mucoadhesive tests showed CMPs have excellent mucosa adhesion for the colonic mucosa of rats. *In vivo* treatment effect of enteric microparticulates systems was evaluated in colitis rats. The results showed superior therapeutic efficiency of this drug delivery system for the colitis rats induced by TNBS. Therefore, the enteric microparticulates systems combined the properties of pH dependent delivery, mucoadhesive, and control release, and could be an available tool for the treatment of human inflammatory bowel disease.

1. Introduction

The incidence of inflammatory bowel disease (IBD), including ulcerative colitis (UC), and Crohn's disease, has increased rapidly in Asia over the last few decades [1]. As the etiology of IBD is still not well-known, therefore, medical therapy is the mainstay of treatment. Among the therapeutic agents, 5-aminosalicylic acid (5-ASA) is currently used as first-line therapy of IBD [2,3]. Recently, curcumin (CUR), a hydrophobic polyphenol derivative extracted from natural herbal source, which has been widely used as herbal remedies for centuries in China and Southeast Asia [4], has received increasing attention for UC therapy. Importantly, clinical trials have shown that CUR is relatively safe for humans, even when given at a high dose (12 g/day) for 3 months [5].

Although there are many drug for the treatment of IBD, the major challenge is to target the drug specifically to the colonic region of GIT [6]. Colon-specific drug delivery systems (CDDS) has turned out to be a promising approach for the treatment IBD by targeted drug release in colon [7–11].

In several of CDDS, it has drawn large attention on micro/nanocarrier-based delivery systems coating with Eudragit or other polymers to cause drug release in colon [12–14]. Particularly, polysaccharide microparticulates coating with Eudragit-enteric materials to avoid drug release in small intestine has showed an excellent consequence of colon target drug delivery [15]. Eudragit S-100, an anionic polymer synthesized from methacrylic acid and methacrylic acid methyl ester, has a property of pH-dependent solubility. It was insoluble in gastric juice and was efficiently used as coating for microspheres aimed for colonic delivery [16].

Several approaches have been used for CDDS, such as prodrug approach, pH dependent system, multiparticulates system, and nano/microparticulate system. Microparticulates system is one of the best approaches for controlled drug delivery in specific site of inflammation [17]. Especially, interpenetrating blend microparticulates of two or more polymers could be used to target the drug in a desired region for release of anti-inflammatory drugs [18]. However, the microparticulates system is limited due to their short residence time at the site of absorption. This can be improved by coupling bioadhesion characteristics to micropartic-

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ulates and developing mucoadhesive microparticulates. Compared to the stomach and small intestine, the colon is deemed a more suitable site for mucoadhesion due to its thicker mucus layer and lower disruptive colonic motility [19]. The combination of the mucoadhesion concept with CDDS would avail a more efficient colonic targeting for treatment of IBD. Thiolated polymers such as thiolated chitosan, have been developed as a mucoadhesive polymers and shown excellent mucoadhesive properties by forming disulphide bonds with cysteine-rich domains of mucus glycoproteins [20].

In the present study, we have developed thiolated chitosan/alginate composite microparticulates (CMPs) coated by Eudragit S-100, and used for colon-specific combined delivery of 5-aminosalicylic acid and curcumin. The physicochemical properties of this microparticulates systems were characterized. *In vitro* drug release was investigated under the simulated complete GIT condition, and the e*x vitro* mucoadhesive tests were carried out with the colon of rats by everted sac method. In addition, *in vivo* treatment effect of this drug delivery system was evaluated in colitis rats induced by TNBS.

2. Materials and methods

2.1. Materials

Chitosan (deacetylation degree 95.0%, $M_w = 1.06 \times 10^6$) was purchased from Sinopharm Chemical Reagent Co., Ltd. Sodium alginate (ALG, $M_w = 6.8 \times 10^5$) was obtained from Tianjin Guangfu Chemical. 1-Ethyl-3-(3-dime-thyllaminopropyl)-carbodiimide hydrochloride (EDC·HCl), 1-Hydroxybenzotriazole (HOBt), and N-acetyl-L-cysteine (NAC) were supplied by Aladdin Chemistry Co. Ltd. 5-ASA (purity >98.0%), CUR (purity >96.0%) and 2, 4, 6-trinitro-benzene-sulfonic acid (TNBS) were obtained from J&K Scientific Ltd. Other chemicals were analytical grade and were used as received.

2.2. Synthesis of thiolated chitosan

Thiolated chitosan (CS-NAC) was prepared as described by Krauland et al. [21], and the obtained CS-NAC was analyzed with ^{1}H NMR and FTIR to prove the successful synthesis. Data for CS-NAC: ^{1}H NMR (500 MHz, D_2O, TMS, ppm): δ 4.65(H-1), 3.78(H-3,H-4), 3.61(H-5,H-6), 3.05(H-2), 2.78 (—S—CH_2—), 1.94(—$COCH_3$). FTIR (KBr): 1692 (amide I band), 1519 (amide II band), and 1313 (amide III band) cm^{-1}. Thiol group content of CS-NAC was 377.5 $\mu mol\ g^{-1}$ determined by Ellman's tests.

2.3. Preparation of CMPs

CS-NAC/ALG CMPs were prepared by the ionic gelation and the polyelectrolyte complexation. In brief, CS-NAC was dissolved in 1% acetic acid. 5-ASA was added into CS-NAC solution, (3 ml, pH 6). CUR was dissolved in 3 ml of alcohol and then the alcoholic curcumin solution was premixed with CS-NAC solution under mechanically stirring for 10 min to full dissolve curcumin. A specific amount of ALG and TPP were dissolved in deionized water. Subsequently, 3 ml of ALG and TPP solution was added dropwise into the above mixture solution of CS-NAC under constant stirring for 30 min, and microparticulates formed. The obtained CMPs were isolated by centrifugation (12000 rpm, 30 min, and 4 °C), and washed with alcohol and deionized water to remove the curcumin and 5-ASA onto surface of the CMPs. And then the CMPs were then lyophilized for following studies.

2.4. Polymeric coating of CMPs

The CMPs was coated with Eudragit S-100 by emulsion-solvent evaporation technique [22,23]. In brief, the CMPs (5 mg) were dispersed in 2.5 ml of anhydrous ethanol containing Eudragit S-100 (2%, w/v) and then emulsified with 40 ml of light liquid paraffin containing Span 80 (1%, v/v). After dropping of 2 ml anhydrous ethanol, the emulsification was mechanically stirred for 3 h at 1000 rpm. The coated CMPs were collected, rinsed with petroleum ether and dried in hot air oven at 50 °C.

2.5. Characterization of CMPs

2.5.1. Determination of drug entrapment efficiency (EE) and loading efficiency (LE)

An indirect method (measurement of the drugs that were not encapsulated) was used to determine the entrapped drugs in CMPs. The prepared CMPs solution was centrifuged at 12,000 rpm for 30 min and washed with alcohol and deionized water. The supernatant and washings were collected, and the concentration of drugs was determined by HPLC. To determine the drug loading efficiency (LE), a specific amount of drug-loaded CMPs (including coated and uncoated CMPs) were dispersed in 10 ml of ethanol and completely swelled at 37 °C for 24 h. The swollen CMPs were crushed by sanitation, and the mixture was centrifuged at 12,000 rpm to get free drug solution. The supernatant was collected and tested with HPLC. All measurements were performed in triplicate to calculate EE and LE by the following formula:

$$EE(\%) = \frac{\text{Total drug (mg)} - \text{Free drug (mg)}}{\text{Total drug (mg)}} \times 100$$

$$LE(\%) = \frac{\text{Total amount of drug in CMPs(mg)}}{\text{Total amount of CMPs(mg)}} \times 100$$

2.5.2. Scanning electron microscopy (SEM) analysis

The morphology of uncoated CMPs and coated CMPs was characterized by SEM (JOEL, Japan) equipped with secondary electron detector. The samples were coated with gold and examined at an accelerating voltage of 20 kV.

2.5.3. Fourier transforms infrared (FTIR) analysis

The chemical structure of uncoated CMPs and coated CMPs were measured by FTIR (Nicolet 670 FTIR, USA). FTIR spectra of 5-ASA, CUR, CS-NAC, ALG, and Eudragit S-100 were also obtained. The samples were prepared by KBr pellet method and the spectral scanning was conducted in wavelength region between 400 and 4000 cm^{-1}.

2.5.4. Determination of thiol groups' content of CMPs

The content of thiol groups present on CMPs was tested by the method reported previously [24], and then the amount of thiol groups on CMPs was calculated by standard curve method.

2.6. In vitro *drug release*

According to the physiological feature of human GIT (pH values were respectively 1.2, 4.5, 6.8 and 7.4, for stomach, duodenum, and small intestine, and colon), the release of drug from uncoated and coated CMPs evaluated in gradually pH-changing medium simulating the conditions of different parts of GIT. The tests were performed in a constant-temperature shower mixer at 100 rpm at 37 °C with 50 ml of dissolution solution. First, samples were placed in a dialysis bag whose molecular weight cutoff was 3500 Da, and placed in pH 1.2 HCl buffer for releasing at the first hour, and then in pH 4.5 PBS for the next 2 h, following in pH 6.8 PBS for the next 2 h, finally in pH 7.4 PBS until the end of the experiment. After