International Journal of Pharmaceutics 516 (2017) 214–224

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Co-delivery of zinc and 5-aminosalicylic acid from alginate/*N*-succinyl-chitosan blend microspheres for synergistic therapy of colitis

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ARTICLE INFO

Article history:
Received 15 August 2016
Received in revised form 3 November 2016
Accepted 12 November 2016
Available online 13 November 2016

Keywords:
Alginate
N-succinyl-chitosan
Blend microspheres
Co-delivery
Colon-specific

ABSTRACT

The present study developed novel zinc ion cross-linked alginate/*N*-succinyl-chitosan (NSC) blend microspheres (MS) for co-delivery of zinc and 5-aminosalicylic acid (5-ASA) for synergistic therapy of colitis. Physicochemical characterization of blend MS was assessed using scanning electron microscopy (SEM), Fourier transform infrared (FTIR) spectroscopy, and energy dispersive X-ray spectrometer (EDS). *In vitro* release studies demonstrated that blend MS has a pH-dependent release property. Both 5-ASA and zinc have lower release in acid medium and higher release in colonic environment. The therapeutic efficacy of zinc cross-linked blend MS was evaluated using induced-colitis rat models, and showed a superior treatment effect in alleviating inflammation of colitis rats. No systemic toxicity was observed after oral administration of blend MS. Therefore, zinc ion cross-linked alginate/*N*-succinyl-chitosan blend MS appeared to be a good candidate for co-delivery of zinc and 5-ASA to colon, and had great potential application in inflammatory bowel diseases (IBD) treatment.

1. Introduction

The inflammatory bowel disease (IBD) is a group of chronic intestinal inflammatory diseases, mainly including Crohn's disease (CD) and ulcerative colitis (UC). Nowadays, the prevalence and incidence of IBD is increasing, especially in developed countries (Moura et al., 2015). As a result of the etiology and pathogenesis of IBD were not yet fully known, the major therapeutic strategies are to relieve or reduce inflammatory episodes. Conventional therapies for IBD treatment mainly involves aminosalicylates, corticosteroids, antibodies and immunomodulators (Yadav et al., 2016). Aminosalicylates have widely been used as first-line therapy for both the treatment of UC and maintenance of remissions (Clapper et al., 2008). 5-ASA is the most frequently used drug in this class. However, the rapid and extensive absorption of 5-ASA in the upper gastrointestinal tract (GIT), which resulted in lower therapeutic effect and the occurrence of side effects (Lichtenstein and Kamm, 2008), had limited to its application.

In recent years, colon-specific drug delivery systems (CDDS) have been widely investigated for the effective treatment of local colonic diseases such as IBD and colorectal cancer (Kavianinia et al., 2016; Prudhviraj et al., 2015; Cerchiara et al., 2015; You et al., 2015; Hua et al., 2015; Gunter and Popeyko, 2016; Seeli and Prabaharan, 2016; Nour et al., 2016; Wang et al., 2016). CDDS could increase the concentration of drug in colon, and also reduce many adverse reactions from systemic absorption of the drug. Therefore, 5-ASA used to treat IBD in the form of CDDS has also drawn much attention (Karrout et al., 2015; Omwancha et al., 2013; Mura et al., 2011a; Wu and Yao, 2013).

Alginate (ALG) is an anionic polysaccharide that composed of glycosidic units of β-D-mannuronic acid and α-guluronic, which has been widely used in drug delivery systems. Due that alginate could form gel particles in the presence of multivalent cations (e.g. Ca^{2+}, Zn^{2+}, Ba^{2+} and Al^{3+}) by ionic cross-linking, it has been extensively used in CDDS. *N*-succinyl chitosan (NSC) is a water-soluble derivative of chitosan that exhibits several biological properties, has been applied as drug delivery carrier (Mura et al., 2011a, 2011b;

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http://dx.doi.org/10.1016/j.ijpharm.2016.11.036
0378-5173/

Ajish et al., 2016; Kamoun, 2016; Bashir et al., 2016; Mukhopadhyay et al., 2014; Yan et al., 2015)

As well known, alginate and/or NSC were regularly cross-linked by Ca^{2+} to form microspheres, microparticles, and hydrogels for drug release or antimicrobial (Cerchiara et al., 2015; Dai et al., 2008; Vicini et al., 2015; Straccia et al., 2014). However, it was reported that alginate microspheres cross-linked by Ca^{2+} have low drug entrapment efficiency and the release of drug from microspheres was non-controllable (Halder et al., 2005). In this study, zinc ion was used as a cross-linker to develop alginate/NSC drug delivery systems. It can form more compact network between zinc ion and carboxylic groups than the most commonly used calcium ion for more resistance to disruption in upper GIT (Das et al., 2011). In another aspect, zinc is necessary for a variety of physiological and biochemical functions including growth and development, reproduction, and immunity (Fukada et al., 2011). It was reported that zinc deficiency had been observed in IBD patients (Filippi et al., 2006; Vagianos et al., 2007). Moreover, studies also had demonstrated that zinc was beneficial for IBD and has protective effects in the GIT (Di Leo et al., 2001; Luk et al., 2002; Sturniolo et al., 2002; Lodemann et al., 2013; Sivalingam et al., 2011). Therefore, zinc ion plays dual roles in this study. For one thing, it was a cross-linker to form drug-delivery carriers in CDDS; for another thing, it can be used as a 'drug' for treatment of IBD.

The aim of the present study is to develop zinc ion cross-linked alginate/NSC blend MS by emulsification/gelation for colon targeting co-delivery of zinc and 5-ASA for synergistic therapy of colitis. The developed blend MS were characterized by SEM, FTIR, and EDS analyses. Furthermore, *in vitro* release properties of 5-ASA and zinc from blend MS were studied in simulated gastrointestinal conditions. Finally, the therapeutic efficacy of the blend MS was evaluated in colitis rat models induced by 2, 4, 6-trinitro-benzene-sulfonic acid (TNBS). In addition, the systemic toxicity of blend MS was observed after oral administration for rats.

2. Materials and methods

2.1. Materials

Sodium alginate (molecular weight, MW: 68 kDa) was obtained from Tianjin Guangfu Chemical. Chitosan (molecular weight, MW: 106 kDa, degree of deacetylation, DD: 95%) was purchased from Sinopharm Chemical Reagent Co., Ltd., 5-ASA (purity >98.0%), succinic anhydride and 2,4,6-trinitro-benzene-sulfonic acid (TNBS) were obtained from J&K Scientific Ltd. All other chemicals and reagents were analytical grade.

2.2. Synthesis of NSC

NSC was prepared according to a previous report (Mukhopadhyay et al., 2014). The structure of NSC was studied through FTIR and ^{1}H NMR (Fig. S1 in Supplementary information). Finally, the yield of the reaction was about 86% and the substitution degree of NSC was about 0.56 determined according to the reported method (Mukhopadhyay et al., 2013).

2.3. Preparation of the blend MS

The blend MS was prepared using emulsification/gelation. Briefly, NSC and sodium alginate were dissolved in 10 ml of NaOH solution (1%, w/v), then specific amount of 5-ASA was added into the mixture solution to form a homogeneous solution. And then this homogeneous solution was emulsified into 40 ml of isooctane containing Span 80 (2%, v/v) with mechanical stirring for 10 min at 800 rpm. Then 1 ml of $ZnCl_2$ solution was added drop wise under mechanical stirring for 30 min at 800 rpm and the bend MS formed. The obtained bend MS were washed with 0.1% Tween 80 solution 3 times and finally lyophilized at −50 °C. The powdered MS were stored in sealed glass vial in vacuum desiccators.

2.4. Characterization

2.4.1. Morphology and sizes

The shape and surface morphology of the blend MS were characterized with SEM (JOEL, Japan). The average particle size and size distribution of the microspheres were also measured from several SEM images.

2.4.2. Fourier transform infrared (FTIR) analysis

The chemical structure of blend MS was analyzed by FTIR (Nicolet 670, USA). The samples were prepared by KBr pellet method and the spectral scanning was conducted in wavelength region between 400 and 4000 cm^{-1}.

2.4.3. Drug loading efficiency (LE)

To determine the drug loading efficiency (LE), a specific amount of drug-loaded blend MS were allowed to swell completely in 10 ml of phosphate buffer solution (PBS, pH 8.0) for 48 h (37 °C, 300 rpm). The higher pH value and rotation speed were employed to prompt break of blend MS and ensure 5-ASA complete release from the blend MS. The mixture was centrifuged to get the polymeric debris free of drug solution. The clear supernatant was collected and examined with UV–vis spectrometer at 310 nm (Perkin-Elmer Lambda 35, USA). All measurements were performed in triplicate to calculate LE by the following formula:

$$\mathrm{LE}(\%) = \frac{\text{Amount of 5 – ASA in blend MS(mg)}}{\text{Mass of blend MS(mg)}} \times 100$$

2.4.4. Zinc ion evaluation

The chemical elements presented in blend MS were identified by EDS (Octane Ultra, EDAX, USA). To determine the amount of zinc in blend MS, 10 mg of blend MS were placed into 50 ml of PBS (pH 8.0) and completely swelled at 37 °C for 48 h. And then the swollen blend MS were crushed by sanitation. The amount of zinc was determined by the complexometric titration method, which EDTA solution (0.05 M) was used as titrant and eriochrome black-T was regarded as indicator.

2.5. In vitro *drug release*

In order to investigate the release properties of 5-ASA and zinc from blend MS, dried drug loaded blend MS (10 mg) were placed in a dialysis bag whose molecular weight cutoff was 3500 Da, and dispersed in 50 ml of release media with pH of 1.2 (HCl buffer), 4.5, 6.8 and 7.4 (PBS) at 100 rpm at 37 °C, respectively. At predetermined time interval, two samples (3.0 ml) were taken out and the fresh buffer solution was added to maintain a constant volume. After centrifugation at 10000 rpm for 5 min, the released amount of 5-ASA and zinc were determined by UV spectrophotometry and complexometric titration method, respectively. All release tests were performed in triplicate.

2.6. Animal experiments

Male Wistar rats were obtained from laboratory animal center of Lanzhou University, and used to evaluate *in vivo* treatment efficacy and *in vivo* toxicity of blend MS. Animals were housed in animal facility at a temperature of 25 °C under 12 h light/dark cycle control room. Standard feeding and drinking water were provided. All animal experimental protocols were reviewed and approved by