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Preparation and evaluation of colon adhesive pellets of 5-aminosalicylic acid

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ABSTRACT

Oral modified-release delivery systems, such as bio-adhesive one, enable drug delivery to affected regions and minimize the side effects by reducing the systemic absorption. Our aim was to develop colon adhesive pellets of 5-aminosalicylic acid (5-ASA) for the treatment of ulcerative colitis. The core of the pellet was formulated from bioadhesive agents, Carbomer 940 and hydroxypropyl cellulose (HPC), by extrusion/spheronization method and coated with Surelease® as inner layer for waterproof and with Eudragit® S100 as outer layer for pH control. The rat model of ulcerative colitis was used to evaluate the efficiency of our loaded pellets as a drug carrier. Microcrystalline cellulose 101 (PH 301) was found to be the best agent for pellet core. The ratio of CP940 to HPC should be kept as (1:1) to achieve high bioadhesion. When the amount of Surelease® was from 16% to 20% and of Eudragit® S100 was 28%, the dissolution profiles of coated pellets revealed no drug release in the artificial gastric fluid (pH 1.0) within 2 h and less than 10% was released in phosphate buffer (pH 6.0) within 2 h whereas complete dissolution was observed in colonic fluid of pH 7.4 for 20 h. The animal experiment showed that 5-ASA loaded colon adhesive pellets had optimal therapeutic effect. We showed a novel approach to prepare effective bioadhesive pellets as colon targeted drug delivery system.

1. Introduction

5-Aminosalicylic acid (5-ASA) is a potent drug for the treatment of inflammatory bowel disease (IBD) (Di Pretoro et al., 2010), and its anti-inflammatory action thought to be predominantly topical at the site of inflammation. The nature of 5-ASA was reported to be changed by GIT acidic conditions (Lewis, 2003). It is rapidly absorbed from the proximal small intestine and metabolized to acetylated 5-ASA. 90% of the metabolite is excreted by the kidneys which can cause renal toxicity. Therefore, conventional oral dosage forms result on a lower availability of the drug on colon, the site of action (Lewis, 2003), and a higher toxicity.

Oral modified-release delivery systems (OMRDS) can maximize the drug delivery to inflamed tissues and reduce the side effects by minimizing the systemic absorption. Therefore, 5-ASA can be considered as their best candidate. OMRDS enable the use of different approaches to protect the 5-ASA, reduce its absorption in the upper digestive tract and increase its concentration on the colon. The pH dependent concept, bioadhesion, time dependent release, pro-drugs and microflora activated systems are some approaches which were investigated for drug targeting to the appropriate site of action (Tozaki et al., 1997; Marvola et al., 1999).

The pH-dependent concept is often based on methacrylic acid copolymers' coatings that are insoluble in the stomach but are rapidly soluble at pH values ranging between 5.5 and 7.0 (Friend, 2005). Additionally, the use of mucoadhesive polymers in pharmaceutical formulations could advantageously improve the therapeutic efficiency by localizing the drug to the target tissue (Akiyama et al., 1995). It has been shown that the use of CP940 as a mucoadhesive agent resulted in a potent retention of particles at the mucosal surface which contributed to extended residence time in the target region (Li et al., 1998).

In this study, we prepared and characterized novel 5-ASA bioadhesive pellets for colonic delivery with a potential benefit for the treatment of IBD. The pellets were prepared by extrusion/spheronization method and coated by a fluid bed coater, with Surelease® as inner layer for waterproof and Eudragit® S100 as outer layer for pH control. The rat model of ulcerative colitis was prepared by 2,4,6-trinitrobenzene sulfonic acid (TNBS) to evaluate the *in vivo* efficacy of 5-ASA colon adhesive pellets.

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2. Materials and methods

2.1. Materials

5-aminosalicylic acid (5-ASA) was purchased from Zhoushan Chemical Industry Plant (Hangzhou, China). 2,4,6-trinitrobenzene sulfonic acid (TNBS) was purchased from Sigma–Aldrich Co., Ltd., (Shanghai, China). Mesalazine SR Granules (Etiasa®) was purchased from Ethypharm Pharmaceutical Co., Ltd., (Shanghai, China). Triethylcitrate (TEC) was purchased from Aladdin Reagent Co., Ltd., (Nanjing, China). Talc was purchased from Longsheng Huamei Co., Ltd., (Guangxi, China). Microcrystalline cellulose (MCC101) was kindly donated by International Specialty Products Company, USA. Microcrystalline cellulose 101 (PH301) was kindly donated by Asahi Kasei Corporation, Japan. Carbomer 940 (CP940) was kindly donated by Nanjing Will Chemical Co., Ltd., (Nanjing, China). Surelease® was donated by Colorcon Coating Technology Co., Ltd., (Shanghai, China). Eudragit® S100 was kindly donated by Evonik Röhm GmbH, (Darm-stadt, Germany).

2.2. Pellet core preparation

Pellets of different composition (Table 1) were prepared by an extrusion/spheronization method. Dry powder mixture was homogenized in a mortar and then wetted. The plastic mass was fed into one-screw axial extruder (E35T, Ingeborg Pharmaceutical Machinery Co., Ltd., Chongqing, China) operated at speed of 25 Hz to form the extrudate. A die of 1 mm thick with perforations of 0.8 mm in diameter was used. The extrudate was then placed into the spheronizer (E35T, Ingeborg Pharmaceutical Machinery Co., Ltd., Chongqing, China) at speed of 25 Hz for 12 min. The prepared pellets were dried in a fluidized bed dryer (Shenyang Institute of Medical linking drug, Liaoning, China) at 40 °C for 0.5 h.

2.3. Double-coating of pellets

2.3.1. Surelease® coating

Pellets were coated in a fluidized bed coater with Surelease® (18% of total pellets weight). 10 g of pellets, with a diameter of 0.6–0.8 mm, were charged into the chamber equipped with a spray nozzle of 0.8 mm diameter and located in the bottom of fluidized bed. Dispersions were sprayed at a rate of 1.0 g/min under an atomization pressure of 0.1 MPa, fluid air flow rate 25 Hz, inlet air temperature was 40 °C and outlet air temperature was 38 °C. The final pellet coating was achieved after incubation at 40 °C for 2 h in an oven to complete the film formation.

2.3.2. Eudragit® S100 coating

Although Surelease® EC coating sustain the drug release from pellets but it cannot retard the release of 5-ASA in the upper digestive tract. Enteric coating with a material such as Eudragit® S100, as outer layer, can improve the concentration of 5-ASA at the colon. Therefore, Surelease® coating pellets were further coated with an outer layer formed from Eudragit® S100, triethyl citrate (TEC) and talc. 10% (w/w) of TEC and 20% (w/w) of talc, both based on polymer weight, were dissolved in 95% (w/w) ethanol under mechanical stirring (XHF-D stirrer, Biological Technology Co., Ltd., Ningbo, China) for 10 min. Eudragit® S100 was dispersed into the above solution under stirring and stirring continued for 60 min to obtain a final dispersion with 8% (w/w) of the total solid contents. The coating level was set to be 28% of the total pellets weight. Batches of 100 g of pellets were coated using a bottom spray fluidized bed coater and the coating conditions were inlet air temperature 30 °C, outlet air temperature 28 °C, fluid air flow rate 25 Hz, atomizing pressure 0.1 MPa and flow process rate 0.8 g/min. Coated pellets were further fluidized for 15 min and kept on an oven at 40 °C for 2 h.

Table 1
Composition of powder mixture and wetting amount.

Sample	5-ASA[a] (g)	PH-301[b] (g)	CP940[c] (g)	HPC-H[d] (g)	30% NaCl[e] (w/w, g)	Water[f] (g)
S1	35	9	4	2	13.3	5.7
S2	35	9	3	3	10	9
S3	35	9	2	4	6.7	12.3

[a] API in adhesive pellet core.
[b] Forming agent in adhesive pellet core.
[c] Adhesive agent in adhesive pellet core.
[d] Adhesive agent in adhesive pellet core.
[e] Antisticking agent in adhesive pellet core.
[f] Wetting agent in adhesive pellet core.

2.4. Pellet characterization

Pellet size and size distribution were determined prior to the coating process by sieve analysis. Pellet shape, angle of repose, friability and yield were evaluated in uncoated pellets sample fraction with diameter of 0.6–0.8 mm.

2.5. Mucoadhesion test

The mucoadhesive performance of uncoated pellets was assessed, *ex vivo*, on colon mucosa of the rat. The colon was cut longitudinally with 5 cm length and cleaned by phosphate buffer (pH 7.4), placed on a polyethylene support, spread and held in position with the help of pins. Uncoated pellet cores were placed uniformly on the mucosa of the colon. The tissue was then placed in a container maintained at 92.5% relative humidity and room temperature for 20 min to allow the polymer to hydrate and to interact with the glycoprotein and also to prevent the drying of the mucus. After 20 min, the mucosa was washed for 5 min with phosphate buffer (pH 7.4) at the rate of 22 ml/mm using a peristaltic pump (Rao and Buri, 1989). Finally, the number of pellets on the mucosa was calculated. A larger number of the pellets in the mucosa will indicate a better adhesion of the pellets.

2.6. In vitro *drug release*

The release of 5-ASA from coated pellets was determined by using the standard basket dissolution method at a rotation speed of 100 rpm in 900 ml of dissolution medium at 37.0 ± 0.5 °C (National Pharmacopoeia Committee, 2010). The dissolution test was performed with a change in the dissolution medium; started with 0.1 N HCl (pH 1.0) for 2 h followed by phosphate buffer (pH 6.0) for 2 h and finally by phosphate buffer (pH 7.4) for 20 h at 37 ± 0.5 °C. The amount of 5-ASA released from the pellets was determined by UV spectrophotometer at a wavelength of 331 nm. All experiments were performed in triplicate.

2.7. Colitis induction and drug administration in rats

Male Sprague-Dawley rats (250–300 g in weight) were purchased from the experimental animal center (Zhejiang, China). Rats were deprived of food but not of water for 24 h prior to the induction of colitis. Colitis was induced with an ethanolic TNBS solution that was instilled into the colon through a catheter at a dose of 50 mg/kg body weight (Siddiqui et al., 2006). A healthy control group received physiological saline instead of the TNBS solution. Three days after TNBS dosing, rats were randomly divided into 7 groups ($n = 5$) to receive one of the following treatments by intra-gastric administration, the control group; the TNBS group; the Etiasa® group (100 mg/kg); the high-dose group, treated with