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Colon targeted oral drug delivery system based on alginate-chitosan microspheres loaded with icariin in the treatment of ulcerative colitis

Qiang-Song Wang[a,1], Gui-Fang Wang[b,1], Jie Zhou[c,1], Li-Na Gao[c], Yuan-Lu Cui[c,*]

[a] Tianjin Key Laboratory of Biomedical Materials, Institute of Biomedical Engineering, Chinese Academy of Medical Science & Peking Union Medical College, Tianjin 300192, PR China
[b] Baokang Hospital, Tianjin University of Traditional Chinese Medicine, Tianjin 300193, PR China
[c] Key Laboratory of Modern Chinese Medicine, Research Center of Traditional Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin 300193, PR China

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ABSTRACT

In recent years, oral colon specific drug delivery system has been paid more attention in the treatment of inflammatory bowel disease (IBD). As the special pH condition in gastrointestinal tract, the challenge for treatment of IBD was that the colon drug delivery system should endure the low pH in stomach and release drugs quickly in high pH in colon. Icariin with the poor solubility and low bioavailability limited the treatment of many diseases in clinic. In this study, the protective mechanism of alginate-chitosan microspheres loaded with icariin were investigated with trinitrobenzene sulfonic acid (TNBS)/ethanol induced colonic mucosal injury in rats. The results of drug release showed that the icariin loaded into microspheres released only 10% in simulated gastric fluid and a high amount of 65.6% released in simulated colonic fluid. The fluorescence tracer indicated high retention of targeted microspheres more than 12 h in colon. The microspheres loaded with icariin could not only reduce the colonic injury by decreasing the colon mucosa damage index in rats, but also reduce the inflammatory response by reducing the production and gene expression of inflammatory mediators and cytokines in colonic mucosa. All the results indicate that targeted microspheres loaded with icariin could exert the colon-protective effects through reducing the inflammatory response, which would be developed as a potential drug controlled release system for treatment of ulcerative colitis.

1. Introduction

Inflammatory bowel disease (IBD), characterized as a chronic inflammatory disease of the gastrointestinal tract with unknown aetiology, was a common disease in United States and Europe with amount of 3.6 million such as crohn's disease, ulcerative colitis (UC) (Loftus, 2004). A challenge in the therapy of UC is the prevention and reduction of drug-related side effects (Buchman, 2001), however, more difficulty in treatment of UC was that the drugs could not reach to the site of action effectively as the special structure of colon.

In recent years, more attention has been paid on oral colon specific drug delivery system due to the advantages in improving local drug concentration, reducing dosage and side effects. Oral colon specific drug delivery system could enhance the systemic bio-availability of poor absorbed drugs as the long retention time in colon (Apninder et al., 2014). The influencing factor of drugs for transiting through the colon mainly was the eating habits and gastric emptying rate, but the most important was associated with the size of dosage form (Challa et al., 2011). In consideration of these advantages, various strategies and biomaterials have been investigated for oral colon specific delivery system, especially the natural biomaterials (Shukla and Tiwari, 2012), such as polysaccharides, which could be degraded by specific enzymes only present in the colon.

Chitosan, a natural derived polysaccharide, has been widely applied in biomedical fields for tissue engineering (Ji et al., 2011), drug delivery systems (Tronci et al., 2014). Alginate, another natural polysaccharide derived from the marine brown algae (Narayanan et al., 2012), shows a wide range of biomedical applications especially in drug delivery, tissue regeneration as its outstanding natures such as biodegradability, biocompatibility and

* Corresponding author. Research Center of Traditional Chinese Medicine, Tianjin University of Traditional Chinese Medicine, No. 88 YuQuan Road, Nankai District, Tianjin 300193, China.
E-mail address: cuiyl@tju.edu.cn (Y.-L. Cui).
[1] Indicates equal contribution.

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chelating ability (Wu et al., 2013). Furthermore, chitosan could form polyelectrolyte with alginate by electrostatic interactions, which could not only make up for the deficiency of alginate hydrogels but also improve the release performance and reinforce the bioadhesive property (Anal and Stevens, 2005). The polysaccharides are good candidate materials for designing the oral colon specific drug delivery system, which could be degraded by microbial enzymes in the colon as its glycosidic linages. The study showed that the dosage forms using polysacchlarides could successfully transport the drugs to the colon and the drugs could ultimately release in the colon through swelling and biodegrading (Vandamme et al., 2002).

UC is a chronic intestinal inflammation with complex pathogenesis (Mennini et al., 2012), and inflammatory bowel disease was triggered by numerous inflammatory cytokines (Bai et al., 2010; von Lampe et al., 2000). In the inflammatory process, lots of inflammatory cytokines, chemokines and other mediators, such nitric oxide (NO), tumor necrosis factor-α (TNF-α), interleukin 1β (IL-1β) and IL-6 involved in the immune response (Hibbs et al., 1987; Palmer et al., 1988). NO is a major product which is controlled by nitric oxide synthases (NOS), such as inducible NOS (iNOS), endothelial NOS (eNOS) and neuronal NOS (nNOS) (Marletta, 1993). Moreover, highly expressed iNOS could lead to organ destruction in some inflammatory and autoimmune diseases (Kleinert et al., 2004). NO could lead to oxidative burst (Chatterjee et al., 2007), which would inflict endothelial damage (Salvemini et al., 1989). As many enzymes and free radicals damage the colonic mucosa, these are believed to contribute to UC formation. Prostaglandin E_2 (PGE_2) is also another important mediator produced from arachidonic acid metabolites catalyzed by cyclooxygenase (COX)-2 in inflammatory responses (Harris et al., 2002).

Icariin, the main active flavonoid glucoside isolated from Herba epimedii (*Epimedium brevicornum* Maxim.), has been confirmed to have numerous pharmacological activities, such as anti-aging, anti-tumor, and anti-inflammatory effects (Chen et al., 2010; Shi et al., 2014; Tao et al., 2013). However, the poor solubility and low bioavailability of icariin limited the treatment of many diseases in clinic. The administration of drugs by the rectal route is also currently used for treating UC, but it is not effective when the inflammation in the upper of the colon. Thus, a targeted microspheres loaded with icariin was prepared to increase the residence time of icariin in colon, moreover, the protective mechanism of microspheres loaded with icariin on trinitrobenzensulfonic acid (TNBS) induced colonic mucosal injury was investigated in rats.

2. Materials and methods

2.1. Reagents and animals

Icariin (Nanjing Tcm Institute of Chinese Materia Medica, Nanjing, China). Sodium alginate and chitosan (with 90% deacetylation degree, Mw 25–60 kDa) were supplied by Sangon Biotech, Co., Ltd. (Shanghai, China). Liquid paraffin was purchased from Beifang Tianyi Chemical Reagent Company (Tianjin, China). Span-80 and Tween-80 were bought from Sigma-Aldrich Co. (St. Louis, MO, USA). All commercially available solvents and reagents were analytical grade and used without further purification. TNF-α, IL-6, IL-1β rat ELISA Kit were obtained from eBioscience (San Diego, CA, USA). Prostaglandin E_2 Parameter Assay Kit was obtained from R&D Systems (Minneapolis, Minnesota, USA). BCA protein assay kit was obtained from Thermo (San Jose, CA, USA). Mammalian cell lysis kit and UNIQ-10 column Trizol total RNA extraction kit were bought from Sangon Biotech, Co., Ltd. (Shanghai, China). Improm-II Reverse Transcription System was purchased from Promega Corporation (Madison, Wisconsin, USA). FastStart Universal SYBR Green Master (ROX) kit was purchased from Roche (Mannheim, Germany).

Male Sprague-Dawley (SD) rats (weighing 200–220 g) were obtained from Beijing Vital River Laboratories Animal Technology Co., Ltd. (Beijing, China). Male ICR mice (weighing 18–22 g) were purchased from Beijing HuaFuKang Bio-technology Co. Ltd. (Beijing, China). The animals were kept under controlled light (12 h light/12 h dark, lights on at 07:00 a.m.). Ambient temperature and relative humidity were maintained at 24 ± 1 °C and 55 ± 5%, respectively. Animal experiments were performed according to the National Institutes of Health Guide for Care and Use of Laboratory Animals, and the protocols were approved by the Animal Ethics Committee of Chinese Academy of Medical Science & Peking Union Medical College.

2.2. Preparation of microspheres

Alginate-chitosan microspheres were prepared according to the following procedure and emulsification-internal gelation technique has been represented by Batich. (Batich and Vaghefi, 1997) Briefly, put Ca-alginate gel beads into 1% chitosan (w/v) glacial acetic solution at pH = 5.5, and make it disperse into 1% chitosan (w/v) glacial acetic solution (1:8) stirring 30 min. Chitosan-coated alginate microspheres are dispersed in glutaraldehyde solution which was used as the cross-linker, and stirred mildly for 2 h. Then glycine solution (1 M) was used to wash the microspheres for eliminating the residual glutaraldehyde solution under the condition of ice water bath.

2.3. Characterization of microspheres

Environmental scanning electron microscopy (ZEISS EVOLS-15, Germany) was used to compare the surface characteristics of dry microspheres. The average size of microspheres and size distribution curves were determined by using a particle-size analyzer (Mastersizer 2000, Malvern, UK). The FT-IR transmission spectra were recorded on a FTS 3000 spectrophotometer (Bio-Rad, CA, USA) in the wave number range of 4000–500 cm^{-1} using KBr pellets.

2.4. Swelling degree of microspheres

Polymers cross-linked with cross-linking reagents could decrease the swelling ability of the micro-molecular chain, which could also affect the drug loading and release characteristics of polymers (George and Abraham, 2006). The different concentrations of glutaraldehyde cross-linked microspheres were soaked in different phosphate buffer of pH = 1.2, 6.8, 7.4 at 37 ± 0.5 °C. The samples were taken out periodically (0,3,6,24 h) and the excess water on the surface of microspheres was removed with the filter paper. The samples were weighed at various time intervals, and each group was assessed in triplicate. The degree of swelling is calculated by following formula.

$$\text{Degree of Swelling (\%)} = \frac{W_t - W_0}{W_0} \times 100$$

Where W_t is weight of the microspheres at time t and W_0 is initial weight of the microspheres.

2.5. Drug release studies in vitro

The release behavior of icariin loaded in alginate-chitosan microspheres was investigated in gradient pH media with rotating paddles (ZRS-8G, Tianjin University Wireless Factory, Tianjin, China). Hydrochloric acid (HCl) buffer pH = 1.2, phosphate buffer