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Colon-specific delivery of 5-aminosalicylic acid from chitosan-Ca-alginate microparticles

K. Mladenovska [a,*], R.S. Raicki [a,1], E.I. Janevik [b,2], T. Ristoski [c,3], M.J. Pavlova [b,2], Z. Kavrakovski [a,1], M.G. Dodov [a,1], K. Goracinova [a,1]

[a] *Ss. Cyril and Methodious University, Faculty of Pharmacy, Vodnjanska 17, 1000 Skopje, Macedonia*
[b] *Ss. Cyril and Methodious University, Faculty of Medicine, Vodnjanska 17, 1000 Skopje, Macedonia*
[c] *Ss. Cyril and Methodious University, Faculty of Veterinary medicine, Lazar Pop Trajkov, 5-7, 1000 Skopje, Macedonia*

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Abstract

Chitosan-Ca-alginate microparticles for colon-specific delivery and controlled release of 5-aminosalicylic acid after peroral administration were prepared using spray drying method followed by ionotropic gelation/polyelectrolyte complexation. Physicochemical characterization pointed to the negatively charged particles with spherical morphology having a mean diameter less than 9 μm. Chitosan was localized dominantly in the particle wall, while for alginate, a homogeneous distribution throughout the particles was observed. ^{1}H NMR, FTIR, X-ray and DSC studies indicated molecularly dispersed drug within the particles with preserved stability during microencapsulation and in simulated *in vivo* drug release conditions. *In vitro* drug release studies carried out in simulated *in vivo* conditions in respect to pH, enzymatic and salt content confirmed the potential of the particles to release the drug in a controlled manner. The diffusional exponents according to the general exponential release equation indicated anomalous (non-Fickian) transport in 5-ASA release controlled by a polymer relaxation, erosion and degradation. Biodistribution studies of [^{131}I]-5-ASA loaded chitosan-Ca-alginate microparticles, carried out within 2 days after peroral administration to Wistar male rats in which TNBS colitis was induced, confirmed the dominant localization of 5-ASA in the colon with low systemic bioavailability.

Keywords: Chitosan-Ca-alginate microparticles; 5-Aminosalicylic acid; Inflammatory bowel diseases; Controlled release; Colon delivery

1. Introduction

5-Aminosalicylic acid (5-ASA) is an anti-inflammatory drug commonly used in the treatment of Crohn's disease and ulcerative colitis, which may provide protection against the development of colorectal cancer in patients suffering from inflammatory bowel diseases (IBD) (Bernstein et al., 2002; Kuang et al., 2002). It appears that many of the effects of 5-ASA can be explained by inhibition of activation of nuclear Factor-κB (NF-κB), which is a central transcription regulatory factor involved in mediating the initiation and perpetuation of inflammatory processes (Bantel et al., 2000; MacDermott and Richard, 2000; D'Acquisto et al., 2002). 5-ASA was demonstrated to inhibit TNF-α stimulated NF-κB activation, NF-κB nuclear translocation and degradation of inhibitory κBα (Kaiser et al., 1999; Verziji and van Bodegraven, 2003).

Activated NF-κB has been detected in macrophages and epithelial cells of colonic biopsies from Crohn's disease and ulcerative colitis patients (Rogler et al., 1998; Schreiber et al., 1998; Schreiber, 1999; Wahl et al., 1998). In this respect, efficacy of 5-ASA correlates with tissue delivery and therefore factors, such as intestinal metabolism and elimination that affect tissue delivery may be important in determining its efficacy (Zhou et al., 1999a). 5-ASA is rapidly absorbed from the small intestine and there is a little localization of 5-ASA in the colon relative to the small intestine (Zhou et al., 1999b). Oral administration is limited also due to the serious adverse effects, such as hepatitis, blood dyscrasias, pancreatitis, pleuropericarditis and interstitial

* Corresponding author. Tel.: +389 2 3126 032; fax: +389 2 3123 054.
E-mail address: krml@ff.ukim.edu.mk (K. Mladenovska).
[1] Tel.: +389 2 3126 032; fax: +389 2 123 054.
[2] Tel.: +389 2 3112 831; fax: +389 2 3147 203.
[3] Tel.: +389 2 3240 700; fax: +389 2 3114 619.

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nephritis associated with 5-ASA therapy (Loftus et al., 2004).

Three methods have been widely used for targeting of 5-ASA to the colon: a pro-drug concept, enteric coating and/or prolonged release of the drug through semipermeable membrane (Prakash and Markharm, 1999; Clemett and Markham, 2000; Loftus et al., 2004). Controlled release preparations are specifically designed to minimize systemic absorption and to achieve optimum delivery of the biologically active 5-ASA moiety to the distal small intestine and the colon. Thus, relatively high concentrations of free 5-ASA can be achieved in the intestinal lumen without producing systemic exposure and subsequent toxicity. This type of target tissue selectivity is a desirable feature for chemopreventive agents and is a significant advantage when considering drug delivery methods to the mucosal surface of the gut.

For these requirements, we have designed a new microparticulate system consisting of chitosan-Ca-alginate matrix in which 5-ASA was dispersed. The use of chitosan provides great promise due to its mucoadhesive properties and non-solubility at pH values higher than 6.5 that prevail in the jejunum and the ileum of the gut (Wittaya-areekul et al., 2006; George and Abraham, 2006). Thus, chitosan-alginate complex erodes slowly in phosphate buffer at pH values higher than 6.5 and this behaviour leads to suppression of the initial drug release in the upper segments of GIT occurring for uncoated microparticles and controls release in the colon whereas pH value is in the range of 6.5–7.0 (Tapia et al., 2004). In addition, chitosan is degraded by the microflora that is available in the colon (Shin-ya et al., 2001; Sardar et al., 2003).

In the case of IBD, an enhanced uptake of administered particles by neutrophils, natural killer cells, mast cells, and regulatory T cells in the inflamed tissue were observed (Lamprecht et al., 2001a,b). This resulted in accumulation of the carrier system in inflamed area. From this point of view, it is advantageous to administer particles that tend to be attached to the mucus layer. Negatively charged particles may adhere more readily to the inflamed tissue because it has been reported that ulcerated tissues contain high concentrations of positively charged proteins that increase the affinity to the negatively charged substances. This was also confirmed by the study of Bernkop-Schnurch et al. (2001), in which anionic alginate showed more potent mucoadhesion in comparison with the cationic chitosan. Thus, an optimal particle size and polymers distribution for the design of a chitosan-alginate particulate carrier system must be chosen in order to prepare microparticles with high drug content and anti-inflammatory effect. Knowledge of the release profile and biodistribution of 5-ASA is essential to achieve optimal targeting when considering variables that determine release such as intraluminal pH and disease activity, which differ largely in patients with chronic IBD.

Considering above-mentioned, 5-ASA loaded chitosan-Ca-alginate microparticles were prepared using spray-drying method associated by ionotropic gelation/polyelectrolyte complexation. Physicochemical characterization, including microparticle size, morphology, polymers distribution, zeta potential, drug loading and drug–polymers interaction was performed as a function of the preparation procedure. The aim of this study was to investigate the influence of the polymers type on drug release and potential of the microparticles for colon delivery of 5-ASA. For this requirements, dissolution profile *in vitro* and biodistribution of 5-ASA contained in this new microparticulate system was studied after peroral administration to rats in which colonic inflammation was induced.

2. Materials and methods

2.1. Materials

Three types of sodium alginate (LF 10/60, fG 65–75%, viscosity 20–70 mPa s for 1% (w/v) solution; LF 120 M, fG 35–45%; viscosity 70–150 mPa s for 1% (w/v) solution, LF 10/60LS, fG 35–45%; viscosity 20–70 mPa s for 1% (w/v) solution) were purchased from Protanal FMC BioPolymers (Norway). Two types of chitosan with a same deacetylation degree ≥85% (low viscosity 342, viscosity of 1% (w/w) solution in acetic acid 20–100 mPa s, M_w 150 kDa, $(R_G^2)^{1/2}$, 44 ± 5 nm and high viscosity 222, viscosity of 1% (w/w) solution in acetic acid 500–2000 mPa s, 659 kDa, $(R_G^2)^{1/2}$, 65 ± 8 nm) were obtained as a gift from France Chitine (France). 5-ASA was purchased from Fluka Chemie AG (Switzerland). Sodium salt of 2,4,6-trinitrobenzenesulphonic acid (TNBS) was purchased from Sigma–Aldrich, Inc. (Germany), *o*-dianisidine hydrochloride from Sigma–Aldrich, Inc. (USA) and hexadecyltrimethylammonium bromide (HTAB) from Sigma–Aldrich, Inc. (Germany).

Radiolabelling of 5-ASA was performed by Na[^{131}I] obtained from Biointernational (France). 1,2,4,6-Tetrachloro-3α,6α-diphenylglucouryl (IODO-GEN, Biointernational, France) was used as an oxidant. All other reagents were of analytical grade.

2.2. Preparation of microparticles

5-ASA loaded microparticles were prepared with slight modifications of the spray-drying method associated with polymer complexation/gelation (Coppi et al., 2001; Liu et al., 1997). A spray-drying technique was applied to 5-ASA/sodim alginate solutions to obtain spherical particles having a mean diameter less than 10 μm. Namely, aqueous dispersion (30 ml) of alginate (3%, w/w) and 5-ASA (0.5%, w/w), adjusted to pH 7.0 by 0.2 M NaOH, was infused into a spray dryer nozzle unit of Büchi Mini Spray Dryer B-191 (Büchi Laboratorius-Technik AG, Flawil, Switzerland) and continuously sprayed using an automatic infusion/withdrawal pump (model Sonceboz 3.1 A/pH, Switzerland) into 90 ml solution of chitosan (0.25%, w/w) and $CaCl_2$ (2.5%, w/v) in 1% (w/w) acetic acid, which was placed in the apparatus collector.

The conditions of the spray-drying process were: nozzle diameter 0.7 mm, aspirator pressure 100%, atomizer pressure 600 N l h^{-1}, flow rate 10 ml/min, inlet temperature 140 °C, outlet temperature 100 °C. The dispersion of microparticles was collected and they were allowed to harden under stirring (3000 rpm)