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Development and performance evaluation of novel nanoparticles of a grafted copolymer loaded with curcumin

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ABSTRACT

Inflammatory bowel disease (IBD) is an inflammatory condition with mucosal ulceration, edema and hemorrhage of gastrointestinal tract. Curcumin has been shown to mitigate colitis in animal models. However, its usefulness is reduced due to poor pharmacokinetic behavior and low oral bioavailability. To address this, novel pH-sensitive hydrolyzed polyacrylamide-grafted-xanthan gum (PAAm-g-XG) nanoparticles (NPs) loaded with curcumin were prepared for colonic delivery. Optimized nanoparticles (CN20) were spherical, with an average size of 425 nm. A negligible amount of curcumin (≈8%) was released from CN20 NPs in pH 1.2 and 4.5 solutions. When the pH was increased to 7.2, curcumin release was comparatively faster than that observed with pH 1.2 and 4.5 collectively. In pH 6.8 solution, excellent release of curcumin was observed. Highest curcumin release was observed when rat caecal contents were incorporated in pH 6.8 solution, indicating microflora-dependent drug release property of NPs. In acetic acid-induced IBD in rats, curcumin NPs reduced myeloperoxidase and nitrite levels, prevented weight loss and attenuated colonic inflammation. Curcumin was better absorbed systemically in nanoparticulate form with increased C_{max} (~3 fold) and AUC (~2.5 fold) than when delivered as free curcumin. We demonstrate successful development of grafted co-polymeric NPs containing drug suitable for colon targeting.

1. Introduction

The chronic inflammatory bowel disease (IBD), comprising Crohn's disease (CD) and ulcerative colitis (UC), is an inflammatory condition with mucosal ulceration, edema and hemorrhage of gastrointestinal tract [1]. Both CD and UC exhibit a relapsing and remitting pattern with reduced quality of life during exacerbations of disease [2]. The etiology of IBD is still unclear but hypothesized to be due to dysfunction of the mucosal immune response toward the gut flora, autoimmune response to mucosal antigen, genetic or environmental factors [1,3]. Current IBD management strategy aims to induce and/or to maintain remission with medications such as anti-inflammatory drugs, immunosuppressive agents, antibiotics and biological agents, with beneficial clinical effects. However, serious adverse effects, cost, and the requirement for systemic delivery demonstrate the need for therapies with high local efficacy and low systemic toxicity [3–5]. Orally-delivered nanoparticles (NPs) offer the benefits of improved absorption, solubility, encapsulation of lipophilic actives, protection of encapsulated drugs from metabolism and enzymatic degradation, resulting in improved drug stability and bioavailability [6]. NPs preferentially absorbed in inflamed regions of the gut due to a disrupted intestinal barrier represent a promising alternative for IBD treatment over existing drug delivery systems [7]. One such lipophilic active is curcumin, a naturally occurring polyphenol with anti-inflammatory, antioxidative, anticancer and antiangiogenesis effects. Its anti-inflammatory activity is mediated by scavenging free radicals and inhibition of myeloperoxidase, COX-1, COX-2, LOX, TNF-α, IFN-γ, iNOS and NF-κB [6]. Curcumin has been shown to attenuate colitis in animal models [3,8]. However, the poor pharmacokinetic behavior and low oral bioavailability of curcumin reduce its usefulness due to its

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Table 1
Composition of different nanoparticle formulations.

Batch code	Curcumin (mg)	Polymer (mg)	1% w/v PVA (mL)	DCM (mL)	$AlCl_3$ (% w/v)	Vol. of $AlCl_3$ (mL)	Sonication parameters[a] (amplitude/time/pulse)
CN1	5	50	50	3	5	5	60/6/6
CN1 (A)	5	50	50	3	2.5	5	60/6/6
CN2	5	50	50	3	5	5	60/6/6
CN2 (A)	5	50	50	3	2.5	5	60/6/6
CN3	5	75	50	3	5	5	60/6/6
CN4	5	25	50	3	5	5	60/6/6
CN5	5	100	50	3	5	5	60/6/6
CN6	2.5	50	50	3	5	5	60/6/6
CN7	10	50	50	3	5	5	60/6/6
CN8	5	50	25	3	5	5	60/6/6
CN9	5	50	100	3	5	5	60/6/6
CN10	5	50	50	3	2.5	5	60/6/6
CN11	5	50	50	3	10	5	60/6/6
CN12	5	50	50	3	5	2.5	60/6/6
CN13	5	50	50	3	5	10	60/6/6
CN14	5	50	50	3	5	5	80/6/6
CN15	5	50	50	3	5	10	40/6/6

PVA—polyvinyl alcohol; DCM—dichloromethane; $AlCl_3$—aluminum chloride.
[a] Time in minutes, pulse in seconds.

extensive metabolism, poor absorption, limited aqueous solubility and instability [9].

There are limited studies of colon-targeted drug delivery systems (CDDS) based on pH and/or enzyme-mediated drug release for curcumin delivery to the colon in IBD. Previously described formulations include microsponges [10], microspheres [11], solid lipid microparticles [12], cyclodextrin complexes [13] and curcumin-inclusion complex tablets with pH- and enzyme-sensitive polymer coating [14]. While Beloqui et al [7] formulated curcumin-NPs using conventional synthetic poly (lactide-*co*-glycolide) (PLGA) and polymethacrylate (Eudragit® S100), Gugulothu et al. [15] prepared pH-sensitive curcumin-celecoxib-NPs using synthetic Eudragit® S100 polymer. Naturally available polysaccharides may be preferable for colon targeting. Substances such as xanthan gum and guar gum have low toxicity, free accessibility, low cost and biodegradability and are degradable by enzymes in the colon [16]. Furthermore, the diverse structure and water solubility of such polysaccharides makes them ideal materials for the synthesis of grafted polymers [17]. Grafting is a novel technique used for modification of polysaccharide, which combines the properties of each material to create desired properties for drug delivery [17–19]. In our current study, polyacrylamide (PAAm) was grafted onto the backbone of xanthan gum (XG) to obtain a grafted polyacrylamide-grafted-xanthan gum (PAAm-*g*-XG) copolymer. Further hydrolysis of PAAm-*g*-XG copolymer converts the amide ($-CONH_2$) functional group of PAAm to a carboxylic acid (–COOH) group, resulting in a pH-sensitive copolymer [16,20]. As XG can be activated by colonic microbiota [21]. PAAm-*g*-XG copolymer should confer high specificity for colon targeting due to its precise pH-dependent degradation and microbiota-dependent activation. We previously described the development of microspheres using grafted polymers [16,18–20]. Development of grafted polymeric NPs is challenging task due to the complexity of the grafted polymer with respect to solubility, gelation or cross-linking issues; but if NP preparation can be achieved using a grafted polymer (such as PAAm-*g*-XG), then the advantages will be better targetability, solubility, absorption and bioavailability, in comparison with microparticles. The objective of the present study was to develop a PAAm-*g*-XG copolymer NPs incorporating a lipophilic anti-inflammatory drug for delivery to the colon in IBD. Here, we developed curcumin-loaded NPs, and evaluated them *in vitro* and *in vivo* in preclinical studies.

2. Materials and methods

2.1. Materials

Curcumin, polyvinyl alcohol (MW: 30,000–70,000; PVA), hydrogen peroxide (30% w/v; H_2O_2) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma–Aldrich (St Louis, MO, USA). PAAm and XG were procured from Central Drug House (P) Ltd., (New-Delhi, India) and HiMedia Laboratories Pvt., Ltd., (Mumbai, India) respectively. Ammonium persulfate (APS), dichloromethane (DCM), HPLC grade acetonitrile and sodium acetate were obtained from S D Fine Chemicals (Mumbai, India). Mannitol, potassium chloride, sodium hydroxide, glacial acetic acid and hydrochloric acid (HCl) were obtained from Merck Specialities Pvt., Ltd (Mumbai, India). Methanol was procured from Qualigens fine chemicals (Mumbai, India). All other chemicals were of analytical grade and used as received. Vero cell lines (normal green monkey kidney epithelial cells) and HCT116 cell lines were purchased from National Centre for Cell Sciences (Pune, India).

2.2. Synthesis and characterization of PAAm-g-XG copolymer

PAAm-*g*-XG copolymer was prepared by free radical polymerization followed by alkaline hydrolysis. Briefly, XG was allowed to hydrate in double distilled water for 4 h with continuous nitrogen gas purging. PAAm and APS were added to XG solution at 80 °C and allowed to polymerize for 60 min with continuous nitrogen gas purging. After cooling, the obtained product was placed in excess methanol for 24 h to de-water. The product was filtered and dried overnight at 50 °C. Then, the dried product was dissolved in 0.9 M sodium hydroxide (2% w/v) and stirred for 60 min at 75 °C in a thermostatic water bath for hydrolysis to occur. After 60 min, the solution was cooled and poured in an excess volume of methanol; the product obtained after filtration was dried overnight at 50 °C and stored in an airtight container free from moisture [18,20]. The synthesized copolymer was characterized by Fourier transform infrared (FTIR) spectroscopy and Elemental analysis to confirm the grafting reaction and alkaline hydrolysis. FTIR Spectroscopy involved KBr pellet press technique using a Shimadzu FTIR 8300 Spectrophotometer (Shimadzu, Tokyo, Japan) in the wavelength range of 400–4000 cm^{-1}. Elemental analysis with the help of