pH-Sensitive Nanoparticles of Curcumin–Celecoxib Combination: Evaluating Drug Synergy in Ulcerative Colitis Model

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ABSTRACT: Inflammatory bowel diseases, which largely comprise ulcerative colitis (UC) and Crohn's disease, are increasingly posing as a global threat because of the incompetence of the current therapy in the entire patient population. This necessitates the identification of alternative therapeutic molecules or their combinations, which may serve as effective first-line or maintenance therapeutics. In this quest, celecoxib, a selective cyclooxygenase-2 inhibiting nonsteroidal anti-inflammatory agent and curcumin, a natural antioxidant and anti-inflammatory agent, have both been found to be useful in alleviating UC. Furthermore, studies involving their combination have proved synergistic action of these two agents. In the current investigation, we have formulated pH-sensitive nanoparticles of curcumin–celecoxib combination as a potential therapy for UC. Synergistic action of the drug combination, delivery advantages of nanosized carriers, and pH-sensitive nature of the polymer were collectively hypothesized to reduce the overall toxicity and total dose of celecoxib and provide enhanced efficacy for mitigating UC. The hypothesis was confirmed in a UC model in rats, where pH-sensitive nanoparticles of the drug combination were found to be more efficacious than nanoparticles of either drugs or drug/s suspension. Further, the blank nanoparticles did not exhibit any therapeutic effect, thereby confirming efficacy of the drug combination for treating UC. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:687–696, 2014

Keywords: colonic drug delivery; drug delivery systems; drug targeting; nanoparticles; natural products; oral drug delivery; polymeric drug carrier; site-specific delivery; targeted drug delivery

INTRODUCTION

Ulcerative colitis (UC), a chronic inflammatory condition affecting colonic mucosa, represents a major arm of the inflammatory bowel diseases (IBDs) that are progressively emerging as a global threat.[1,2] Although medically incurable, the current therapy for management of this condition comprises of amino salicylates, corticosteroids, immunosuppressive agents, and molecules (biological and synthetic) that target tumor necrosis factor-α (TNF-α), involved in the disease pathogenesis.[3,4] However, the majority of these drugs are effective in only half of the total patient population, leaving the other half exposed to high probability of disease relapse and need for prolonged maintenance therapy.[4,5] This also calls for additional research to identify therapeutic molecules or their combinations, which could serve as alternative first-line or maintenance therapy for UC.

In this regard, celecoxib, the nonsteroidal anti-inflammatory drug that inhibits the enzyme cyclooxygenase-2 (COX-2), is being looked upon as an interesting option. COX-2 is involved in the production of prostaglandins, which are mediators of pain and inflammation. Thus, selective inhibition of this enzyme has been found to alleviate gastric ulceration and bleeding. Preliminary studies have indicated the safety and efficacy of celecoxib during short-term therapy of IBD patients.[6] However, no comprehensive studies discussing employment of celecoxib for IBD have been reported till date, to the best of our knowledge.

Another molecule that has caught the attention of research scientists, for application in IBD, includes curcumin—the anti-inflammatory constituent of turmeric—the herbal spice. The anti-inflammatory activity of this agent has been attributed to its ability to inhibit COX-2 and inflammatory cytokines such as interleukines-1 β and -6 and TNF-α. Because of this action, curcumin has been reported to reduce mucosal injury in murine models of UC and has exhibited efficacy in some clinical investigations of IBD.[7,8] Another particular advantage of curcumin is its long-term safety, even at high doses, which may be suitable for maintenance therapy in IBD.[9]

In addition to the studies showing individual potential of celecoxib and curcumin, researchers have also studied the synergistic action of both these molecules because of their different mechanisms of inhibiting COX-2. Studies conducted in colorectal cancer cells and osteoarthritis synovial adherent cells confirmed that curcumin could augment the inhibition of COX-2 by celecoxib. These investigations suggested the possibility of employing lower and safer doses of celecoxib as an anti-inflammatory agent, [10,11] this outcome being significant for diseases requiring prolonged drug administration, as in the case of IBD. Additional advantage of using this combination is the ability of curcumin to simultaneously inhibit 5-lipoxygenase (5-LOX) and COX.[12] It has been previously reported that such concomitant inhibitors of COX and 5-LOX could lead to better inhibition of leukotrienes and prostaglandins, both of which contribute toward the development of inflammatory disorders.[13] This may further reduce the dose of celecoxib that may be required for anti-inflammatory action. Furthermore, curcumin is a proven gastro protective agent, which strengthens the gastric mucosa by reduction in acid secretion, its antioxidant, and antiapoptotic effects.[14] Thus, coadministration of gastroprotectant along with selective COX-2 inhibitor such as celecoxib is likely to reduce the gastric adverse effects of celecoxib.[15] However, so far, no investigation

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has explored the combination of these two agents for mitigating IBD.

Despite the preliminary proof of efficacy for treating IBD, many of the recently identified molecules are not completely effective because of a myriad of reasons including limited solubility, poor bioavailability, nonspecific tissue distribution and related side effects, poor absorption and rapid elimination, poor retention in colon, and so on. However, many of these problems have been overcome by encapsulating the drugs into nanoparticulate carriers.[9,16]

Specifically concerning curcumin, our research group has previously reported nanoparticles of this agent using a pH-sensitive enteric polymer, nontoxic polymer (Eudragit® S100). This polymer dissolves at the pH 7.0 by ionization of its carboxylic functional group and thus has been used for targeting drugs to the colonic region of gastrointestinal tract (GIT). These nanoparticles were found to be more effective than unencapsulated drug in cellular assays.[9]

In this investigation, we have formulated Eudragit® S100 nanoparticles loaded with curcumin–celecoxib combination and studied their efficacy in trinitrobenzene sulfonic acid (TNBS)-induced UC model in rats. Along with the aforementioned benefits of the drug combination in reducing celecoxib-related adverse effects, selective targeting to the colon with the chosen polymer, was hypothesized to overcome the renal and cardiotoxicity of celecoxib.[17] Nanoparticles of individual drugs, formulated with the same polymer, were used as controls. Nanoparticles of the drug combination were found to exhibit superior action as compared with the nanoparticles of individual drugs or suspension of drug combination. This may be collectively attributed to size-influenced preferential uptake of nanoparticles, selective targeting to colon, and synergistic effect of the encapsulated drugs. Further, this synergistic action along with improved drug bioavailability imparted by the nanosized carriers may significantly reduce the total dose of celecoxib. Overall, the pH-sensitive nanoparticles of this drug combination may provide an effective therapy for IBD.

EXPERIMENTAL

Materials

Curcumin (Cur; assay: 95%) and celecoxib were gift samples from Konark Herbals and Healthcare Pvt. Ltd. (Mumbai, India) and Cadila Pharmaceuticals Ltd. (Ahmedabad, India), respectively. Eudragit® S100 (Rohm Pharma) was provided by Evonik Degussa India Pvt. Ltd. (Mumbai, India). Poly vinyl pyrrolidone (PVP K-90/D) was procured from ISP Technologies Inc. (Wayne, New Jersey). Poly vinyl alcohol, (87%–90% hydrolyzed, molecular weight: 30,000–70,000 Da) was purchased from Sigma–Aldrich (Mumbai, India). TPGS (d-tocopheryl polyethylene glycol1000 succinate) from Eastman Chemical Company (Kingsport, Tennessee) and Pluronic® F68 [Poloxamer 188 N.F., poly (ethyleneoxide)–poly (propylene oxide) block copolymer] from BASF India Ltd. (Mumbai, India) were gift samples. HPLC grade methanol and dichloromethane and AR grade acetone were purchased from Rankem (Mumbai, India). Trehalose was obtained as a gift sample from Gangwal Chemicals Pvt. Ltd. (Mumbai, India). Sucrose was purchased from s.d. Fine Chemicals (Mumbai, India). Deionized, double distilled water (Milli-Q-Plus system; Millipore, Bedford, Massachusetts) was used throughout the study.

Methods

Preparation of Cur-, Celecoxib-, and Cur–Celecoxib-Loaded Eudragit®S100 Nanoparticles

All the nanoparticles were prepared using solvent emulsion evaporation technique, as published previously.[9]

For preparing curcumin-loaded nanoparticles (CurNPs), curcumin (10 mg) and Eudragit® S100 (10 mg) were dissolved in acetone (2 mL). The organic solution was emulsified with aqueous solution (20 mL) containing TPGS (0.05%, w/v) and PVP (0.075%, w/v) using Ultraturrax® T25 (Janke and Kunkel, IKA Labortechnik, Staufen, Germany) at 17,500 rpm for 5 min. The organic phase was subsequently evaporated at 27°C, using a blade-type stirrer (Remi, Mumbai, India), at 2000 rpm for 8 h to formulate the nanoparticles. In case of celecoxib (Cel)-loaded nanoparticles (CelNPs), the organic phase consisted of a mixture of celecoxib (10 mg) dissolved in dichloromethane (1 mL) and Eudragit® S100 (15 mg) dissolved in acetone (3 mL). The remaining procedure was identical to that followed for CurNPs. Nanoparticles of combination of Cur–Cel were also prepared by the same technique. However, in this case, the organic phase comprised a mixture of curcumin (10 mg) and Eudragit® S100 (10 mg) dissolved in acetone (2 mL) and celecoxib (1 mg) dissolved in dichloromethane (1 mL). Although the surface-active agents used in aqueous phase (20 mL) consisted of TPGS (0.125%, w/v) and PVP (0.150%, w/v).

Nanoparticle Characterization

Particle Size and Surface Charge

The average particle size, formulation homogeneity in terms of polydispersity index (PI) and surface charge in terms of zeta (ζ) potential were determined using Zetasizer Nano-ZS (Malvern Instruments, Worcestershire, UK) equipped with a 4-mW He–Ne laser. Measurements were conducted in triplicates, at 25°C, employing a wavelength of 633 nm and a backscattering angle of 173°. All the samples were sufficiently diluted with Milli-Q water to ensure that their light scattering intensity was within the instrument's sensitivity range.

Encapsulation Efficiency

Encapsulation efficiency (EE) of nanoparticles indicates the amount of drug incorporated within the nanoparticles as compared with the total drug added to the formulation. EE of all the nanoparticle suspensions was determined by ultracentrifuging the formulations (2 mL) at room temperature, at the speed of 70,000 rpm (450000 × g) for 40 min. The amount of unencapsulated drugs in the supernatant was analyzed by a validated HPLC method.[18] The experiments were performed in triplicate. The EE (%) was calculated by the following equation:

$$\mathrm{EE}(\%) = \frac{M_{\text{initial drug}} - M_{\text{free drug}}}{M_{\text{initial drug}}} \times 100$$

where "$M_{\text{initial drug}}$" is the mass of total drug used for the formulation and "$M_{\text{free drug}}$" is the mass of unencapsulated drug analyzed in the supernatant after centrifugation.

Freeze-Drying of Nanoparticles

Freeze-drying was carried out using a laboratory freeze-dryer (Freezone12, Labconco, Kansas city, Missouri) on the frozen nanoparticles formulation (−40°C, 12 h). The cryoprotectants