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Wheat germ agglutinin anchored chitosan microspheres of reduced brominated derivative of noscapine ameliorated acute inflammation in experimental colitis

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ABSTRACT

Reduced brominated derivative of noscapine (Red-Br-Nos, EM012), has potent anti-inflammatory property. However, physicochemical limitations of Red-Br-Nos like low aqueous solubility (0.43×10^{-3} g/mL), high lipophilicity (log$P \sim 2.94$) and ionization at acidic pH greatly encumber the scale-up of oral drug delivery systems for the management of colitis. Therefore, in present investigation, chitosan microspheres bearing Red-Br-Nos (CTS-MS-Red-Br-Nos) were prepared by emulsion polymerization method and later coated with wheat germ agglutinin (WGA-CTS-MS-Red-Br-Nos) to boost the bioadhesive property. The mean particle size and zeta-potential of CTS-MS-Red-Br-Nos were measured to be 10.5 ± 5.4 μm and 8.1 ± 2.2 mV, significantly ($P < 0.05$) lesser than, 30.2 ± 3.2 μm and 19.2 ± 2.3 mV of WGA-CTS-MS-Red-Br-Nos. Furthermore, various spectral techniques like SEM, FT-IR, DSC and PXRD substantiated that Red-Br-Nos was molecularly dispersed in tailored microspheres in amorphous state. Surface bioadhesive property of WGA-CTS-MS-Red-Br-Nos promoted the affinity toward colon mucin cells in simulated colonic fluid (SCF, pH ~ 7.2). *In vitro* release studies carried out on WGA-CTS-MS-Red-Br-Nos and CTS-MS-Red-Br-Nos indicated that SCF with colitis *milieu* (pH ~ 4.7) favored the controlled release of Red-Br-Nos, owing to solubilization at acidic pH. Consistently, *in vivo* investigation also demonstrated the utility of WGA-CTS-MS-Red-Br-Nos, which remarkably attenuated the DSS encouraged neutrophil infiltration, myeloperoxidase activity, and pro-inflammatory cytokine production in C57BL6J mice, as compared to CTS-MS-Red-Br-Nos and Red-Br-Nos suspension. The noteworthy anti-inflammatory activity of WGA-CTS-MS-Red-Br-Nos against acute colitis may be attributed to enhanced drug delivery, affinity and utmost drug exposure at inflamed mucosal layers of colon. In conclusion, WGA-CTS-MS-Red-Br-Nos warrants further in-depth *in vitro* and *in vivo* investigations to scale-up the technology for clinical translation.

1. Introduction

Colitis is associated with recurring episodes of inflammation at the mucosal layer of colon [1,2]. Current therapeutic regimen for colitis includes 5-aminosalicylates, corticosteroids and/or immunomodulators [3,4]. However, reported major side-effects of anti-inflammatory drugs in gastrointestinal tract are diarrhea, nausea, headache, tingling of hands or feet and increased blood pressure [5]. Noscapine, a plant-derived, non-toxic alkaloid exhibited both tubulin-binding and anti-inflammatory properties [6–8]. Moreover, a reduced brominated derivative of noscapine, Red-Br-Nos (EM012) was also about 5–40 fold more potent than the parent compound, noscapine [9]. Red-Br-Nos inhibits toll-like receptors (TLRs), tumor necrosis factor-α (TNF-α) and nitric oxide (NO) release in human and murine macrophages without evidence of cellular toxicity. Mechanistically, Red-Br-Nos exhibits anti-inflammatory effect either by interfering with target cells or invalidating the signal transduction by attenuating microtubule dynamics or by inducing autophagy cell death [10]. However, physicochemical limitations of Red-Br-Nos like low aqueous

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solubility (0.43×10^{-3} g/mL), high lipophilicity ($\log P \sim 2.94$) and ionization at acidic pH greatly encumber the scale-up of oral drug delivery systems for the management of colitis [11]. Solubility, bioavailability and controlled release pattern are prerequisite for efficient delivery of anti-inflammatory drugs through oral route of administration for the management of colitis. This consequently prevents premature drug degradation and localizes the drug molecules at the target site [12,13].

Recently, chitosan microspheres (CTS-MS) have been extensively investigated for the oral delivery of anti-inflammatory drugs to colon [14–16]. CTS-MS promote bioadhesion at mucus layer and consequently drug delivery at the site of action [17]. In addition, modification of the microspheres with lectin further enhanced the bioadhesion and residence time of particulate systems in colon [18,19]. In this series, wheat germ agglutinin (WGA), a non-toxic and least immunogenic glycoprotein has shown affinity for the components of mucosal layer, N-acetyl-D-glucosamine and sialic acid residue [13]. Moreover, WGA endorsed the robustness of particulate system against acidic pH and enzymatic degradation [20].

Therefore, in present investigation, wheat germ agglutinin conjugated chitosan microspheres of Red-Br-Nos (WGA-CTS-MS-Red-Br-Nos) were prepared using emulsion polymerization technique [21]. Chitosan microspheres of Red-Br-Nos (CTS-MS-Red-Br-Nos) were also prepared for comparative studies. In addition, various characterization parameters like particle size, zeta-potential, encapsulation efficiency, bioadhesive property and *in vitro* drug release were examined using analytical and spectral techniques. The *in vivo* study was conducted to analyze the anti-inflammatory activity of WGA-CTS-MS-Red-Br-Nos, CTS-MS-Red-Br-Nos and Red-Br-Nos suspension in dextran sodium sulfate (DSS) induced experimental colitis model [22].

2. Materials and methods

2.1. Chemicals and reagents

Red-Br-Nos, EM012 [(R)-9-bromo-5-((S)-4,5-dimethoxy 1,3-dihydroisobenzofuran-1-yl)-4-methoxy-6-methyl-5,6,7,8-tetrahydro-1,3-dioxolo-[4,5-*g*]-isoquinoline] was synthesized by our group [23]. Wheat germ agglutinin (lyophilized powder) was purchased from Sigma–Aldrich, USA. Chitosan (molecular weight ~1,50,000 Da; 75–85% deacetylated) was procured from Himedia Limited, Mumbai, India. Light liquid paraffin, heavy liquid paraffin, glutaraldehyde (25% v/v aqueous solution) and span 80 were obtained from Loba Chemie, New Delhi, India. Dextran sodium sulfate (DSS, molecular weight ~36000–50,000, Colitis grade) was purchased from MP Biomedical, Solon, OH. All other chemicals used were of highest analytical grade.

2.2. Preparation of chitosan microspheres

Chitosan microspheres encapsulating reduced bromonoscapine (CTS-MS-Red-Br-Nos) were prepared by emulsion polymerization method with minor modifications [21]. In brief, oil phase was prepared by mixing 75 mL of light liquid paraffin with 75 mL of hard liquid paraffin in a 250 mL beaker. Subsequently, 1% w/w of span 80 was added to the oil phase and maintained at 70 °C. To this oil phase, 2% w/v chitosan gel (prepared by mixing 200 mg of chitosan and 0.2 mL of 17.4 M acetic acid solution in 10 mL of water) containing 50 mg of Red-Br-Nos was added drop-wise using hypodermic syringe. The emulsion was stirred at 4000 rpm at 70 °C for 1 h. After stirring, a saturated solution of cross-linking agent (10 mL glutaraldehyde: 30 mL of toluene) was added to the emulsion and stirred again for next 1 h at 55 °C. The cross-linked microspheres, CTS-MS-Red-Br-Nos were separated by filtration and washed several times with *n*-hexane. The product was dried at room temperature and stored in a desiccator until further use.

2.3. Conjugation of wheat germ agglutinin onto the chitosan microspheres

Wheat germ agglutinin anchored chitosan microspheres of reduced bromonoscapine (WGA-CTS-MS-Red-Br-Nos) were prepared by conjugating WGA on to the surface of CTS-MS-Red-Br-Nos using covalent-coupling technique [24]. In brief, 100 mg of CTS-MS-Red-Br-Nos was added to 3 mL of 0.75% w/v EDAC·HCl solution in phosphate buffer (pH ~ 6.0) at room temperature to activate the microspheres. The mixture was vortexed for 30 min, followed by dialysis against distilled water to remove un-reacted components. The activated microspheres were then transferred to 3 mL of phosphate buffer (pH ~ 6.0) containing 5 mg of WGA and incubated for 30 min. The microspheres were centrifuged at 4000 rpm and supernatant liquid was collected and stored at 4 °C until further analysis. Finally, WGA-CTS-MS-Red-Br-Nos were collected and stored in a dessicator. Conjugation was confirmed using Fourier-transforms infrared (FT-IR) spectroscopy. The spectrum of CTS-MS-Red-Br-Nos and WGA-CTS-MS-Red-Br-Nos was captured using spectrum BX (Perkin Elmer, Massachusetts, USA) infrared spectrophotometer. Samples were prepared separately in KBr pellets, using a hydrostatic press at a force of 40 psi for 4 min. Each sample was scanned thrice between 400 and 4400 cm^{-1} at a resolution of 4 cm^{-1} and averaged for that sample and there were three ($n = 3$) different samples scanned.

2.4. Determination of binding capacity of wheat germ agglutinin on to the surface of chitosan microspheres

The free amine groups in total amount of WGA added and unconjugated WGA in supernatants were determined by TNBS (Trinitro benzene sulfonic acid) assay [25]. Briefly, both samples in equal amount were mixed separately with 1 mL of 4% w/v sodium bicarbonate (pH ~ 8.5) and 1 mL of 0.1% v/v TNBS, respectively. Mixtures were then kept in an incubator for 4 h at 40 °C in dark with mild shaking. Subsequently, 3 mL of 6 N HCl was added separately to the reaction mixture to hydrolyze any insoluble material and heated at 70 °C for 1 h. The hydrolysate was diluted with distilled water (5 mL) and extracted with diethyl ether. A volume of 5 mL of the aqueous phase was removed from each sample and heated for 15 min, cooled to room temperature, and diluted with 15 mL of distilled water. Absorbance was measured at 340 nm in an UV–Visible spectrophotometer (1800, Shimadzu, Kyoto, Japan) against a blank. The experiment was carried out in triplicate ($n = 3$).

The binding of WGA to CTS-MS-Red-Br-Nos was calculated as:

$$\%\ \text{Amine groups modified} : \frac{\text{Absorbance of total WGA added} - \text{Absorbance of supernatant WGA}}{\text{Absorbance of total WGA added}} \times 100$$

2.5. Characterization of microspheres

2.5.1. Particle size and zeta-potential

Particle size and zeta-potential of microspheres was determined by Master-sizer (Malvern Instrument, UK). Briefly, 5 mg quantity of each sample was suspended separately in 1 mL of simulated colonic fluid (SCF, pH ~ 7.2 without enzymes). Each sample was then transferred to a cuvette to determine the particle size and zeta potential. A 150 mV electric field was employed to determine the electrophoretic velocity of the microspheres. Results were depicted as mean value ($n = 3$) ± SD.