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Edible ginger-derived nanoparticles: A novel therapeutic approach for the prevention and treatment of inflammatory bowel disease and colitis-associated cancer

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ABSTRACT

There is a clinical need for new, more effective treatments for chronic and debilitating inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis. In this study, we characterized a specific population of nanoparticles derived from edible ginger (GDNPs 2) and demonstrated their efficient colon targeting following oral administration. GDNPs 2 had an average size of ~230 nm and exhibited a negative zeta potential. These nanoparticles contained high levels of lipids, a few proteins, ~125 microRNAs (miRNAs), and large amounts of ginger bioactive constituents (6-gingerol and 6-shogaol). We also demonstrated that GDNPs 2 were mainly taken up by intestinal epithelial cells (IECs) and macrophages, and were nontoxic. Using different mouse colitis models, we showed that GDNPs 2 reduced acute colitis, enhanced intestinal repair, and prevented chronic colitis and colitis-associated cancer (CAC). 2D-DIGE/MS analyses further identified molecular target candidates of GDNPs 2 involved in these mouse models. Oral administration of GDNPs 2 increased the survival and proliferation of IECs and reduced the pro-inflammatory cytokines (TNF-α, IL-6 and IL-1β), and increased the anti-inflammatory cytokines (IL-10 and IL-22) in colitis models, suggesting that GDNPs 2 has the potential to attenuate damaging factors while promoting the healing effect. In conclusion, GDNPs 2, nanoparticles derived from edible ginger, represent a novel, natural delivery mechanism for improving IBD prevention and treatment with an added benefit of overcoming limitations such as potential toxicity and limited production scale that are common with synthetic nanoparticles.

1. Introduction

Inflammatory bowel diseases (IBDs), which include ulcerative colitis and Crohn's disease, are chronic, debilitating inflammatory conditions for which existing treatments are largely limited by serious systemic side effects [1–3]. Over the last decade, the treatment options for IBD have been anti-inflammatory medications (5-amino salicylic acid, steroids) or immunosuppressants [4–6]. Despite the efficacy of these medications, further applications are limited by their non-specific actions on immune system that result in short- and long-term debilitating side effects, such as allergic reactions, nausea, elevated liver tests, pancreatitis, and other life-threatening side effects [7]. Furthermore, anti-inflammatory drugs that are locally active with minimal systemic absorption (5-aminosalicylates) require frequent high-dose administration to exert measurable clinical efficacy. Moreover, while sustained drug-release devices, such as pellets, capsules or tablets, designed to deliver drugs specifically to the colon for longer

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periods of time have been developed, these drugs have limited therapeutic efficacy and are effective in only a subset of IBD patients [8–13].

More recently, targeted therapeutic approaches, based on the pathophysiology of inflammatory responses in IBD, have been developed. These therapeutic strategies can be divided into three categories: development of inhibitors of inflammatory cytokines, such as tumor necrosis factor (TNF)-α, that induce T-lymphocyte apoptosis; identification of anti-inflammatory cytokines that down-regulate T-lymphocyte proliferation; and synthesis of selective adhesion molecule inhibitors that suppress T-lymphocyte trafficking into the gut epithelium. Anti-TNF-α agents are among the most potent drugs in the treatment of IBD. However, they must be administered systemically and their use is limited by serious side effects [14]. Thus, there is an unmet need for a carrier system capable of delivering drugs specifically and exclusively to the inflamed regions for a prolonged period of time. Such a system could significantly reduce the side effects of existing, otherwise effective, treatments.

To address this formidable challenge, targeting drug carriers based on nanoparticles have been designed and have shown great promises for improving IBD treatment. Various carriers have been designed to release the drug at a specific pH value, to be resistant to digestive enzymes, and/or require bacterial cleavage for activation, in which several of these carriers are currently being investigated. Our laboratory and others have recently demonstrated that artificially synthesized nanoparticles can be used to deliver low doses of drugs to specific cell types and tissues, and decrease the systemic side effects of medications [15–26]. However, the nanoparticles synthesized to date have two major limitations: i) each constituent of the synthesized nanoparticles must be examined for potential *in vivo* toxicity before clinical application; and ii) the production scale is limited. In contrast, nanoparticles derived from natural sources are considered to be safe and cost effective that may overcome aforementioned limitations of synthetic nanoparticles [27]. Recently, exosome-like nanoparticles isolated from edible plants using an eco-friendly protocol have been characterized [28]. These nature-derived nanoparticles could serve interspecies communication roles and exert anti-inflammatory properties in IBD treatment [29–31]. These observations suggest that the application of plants as “nanofactories” for the fabrication of medical nanoparticles could represent a new approach for IBD treatment.

Ginger, the rhizome of *Zingiber officinale*, is one of the most widely used natural products. It is consumed as a spice and used as a medicine for the treatment of nausea, as well as other digestive tract problems like colic, flatulence, diarrhea and dyspepsia [32–35]. Studies have also shown that ginger and its active components, including 6-gingerol and 6-shogaol, exert anti-oxidative, anti-inflammatory, and anti-cancer activities [36–38]. In the present study, we assessed the feasibility of isolating ginger-derived nanoparticles, characterized their properties, and examined their potential use as a new treatment for IBD and CAC.

2. Materials and methods

2.1. Chemicals

The fluorescent lipophilic dyes, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiL), 3,3′-dioctadecyloxacarbocyanine perchlorate (DiO) and 1,1′-dioctadecyl-3,3′,3′-tetramethylindotricarbocyanine iodide (DiR), were purchased from Promokine (Heidelberg, Germany); DC-Chol/DOPE Blend was from Avanti Polar Lipids (Alabaster, AL, USA); phalloidin-FITC, O-dianisidine dihydrochloride, myeloperoxidase from human leukocytes, type VIII collagenase, DNase I, (6)-gingerol and (6)-shogaol standards were purchased from Sigma (St. Louis, MO, USA). Rabbit anti-mouse E-cadherin antibody was from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-mouse CD326 (EpCAM) PE-Cy7, anti-mouse CD11b eFluo 450; anti-mouse CD11c APC, and anti-mouse F4/80 antigen PE-Cy7 were purchased from eBioscience (San Diego, CA, USA). Duoset enzyme-linked immunosorbent assay (ELISA) kits were purchased from R&D Systems (Minneapolis, MN, USA).

2.2. Isolation, purification, and characterization of ginger-derived nanoparticles (GDNPs)

For isolation of GDNPs, ginger or Zingiber officinale (Family, Zingiberaceae; Order, Zingiberales; Superorder, Lilianae; Subclass, magnoliidae; Class, Equisetopsida) was purchased from a local farmers' market. The utilized ginger was purchased from three different farmers' markets in Atlanta, Georgia; More than 20 batches were purchased over the past 12 months. Results similar to those reported in the manuscript were obtained using these different ginger batches from different sources. Ginger was washed thoroughly with tap water at room temperature (22 °C). After the final washing, the ginger was ground in a blender to obtain juice, then the juice was centrifuged first at 3000*g* for 20 min and then at 10,000*g* for 40 min to remove large ginger fibers. The supernatant was ultracentrifuged at 150,000*g* for 2 h, and the pellet was suspended in phosphate-buffered saline (PBS) through ultrasonic dispersion.

For purification of GDNPs, the suspension was transferred to a discontinuous sucrose gradient (8%, 30%, 45% and 60% [g/v]) and ultracentrifuged at 150,000*g* for an additional 2 h. The bands between 8/30%, 30/45%, and 45/60% layers, which corresponds to GDNPs 1, GDNPs 2 and GDNPs 3, respectively, were harvested. The concentrations of the GDNPs obtained were quantified based on protein concentration using a Bio-Rad protein quantification assay kit. The quantified GDNPs were stored at −80 °C until use.

GDNPs were characterized with respect to size and zeta potential by dynamic light scattering using 90 Plus/BI-MAS (multi-angle particle sizing) or dynamic light scattering after applying an electric field using a ZetaPlus instrument (Brookhaven Instruments Corp, Holtsville, NY, USA). Atomic force microscopy (AFM) images were acquired using an SPA 400 AFM instrument (Seiko Instruments Inc., Chiba, Japan). For transmission electron microscopy (TEM) imaging, a drop of sample was deposited onto the surface of a formvar-coated copper grid, after which 1% uranyl acetate was added for 15 s and the sample was allowed to dry at room temperature for subsequent imaging.

For *in vitro* stability tests, 1.34 μl of 18.5% (w/v) HCl (pH 2.0) and 24 μl of pepsin solution (80 mg/ml in 0.1 N HCl, pH 2.0) were added to 1 ml (1 mg/ml) of GDNPs in PBS, and the mixture was incubated at 37 °C for 0.5 h (stomach-like conditions). Then, 80 μl of a mixture containing 24 mg/ml of bile extract and 4 mg/ml of pancreatin in 0.1 N $NaHCO_3$ was added. The pH was adjusted to 6.5 with 1 N $NaHCO_3$ and incubated for an additional 0.5 h under the same conditions (intestine-like). The stability of GDNPs was evaluated by measuring particle size and zeta potential using the method described above.

2.3. Lipids, proteomics, and microRNA sequencing discovery of GDNPs

For lipidomic analyses, lipid samples extracted from band 1, 2 and 3 were submitted to the Lipidomics Research Center, Kansas State University (Manhattan, KS, USA) for analysis. Briefly, the lipid composition of GDNPs was determined using a triple quadrupole