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Preventive effects of Goji berry on dextran-sulfate-sodium-induced colitis in mice

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Abstract

Goji berry (*Lycium barbarum*) exerts immune modulation and suppresses inflammation *in vitro* and *in vivo*. We hypothesized that Goji berry had beneficial effects on dextran sulfate sodium (DSS)-induced colitis in C57BL/6 mice through suppressing inflammation. Six-week-old male C57BL/6 mice were supplemented with a standard AIN-93G diet with or without 1% (w/w) Goji berry for 4 weeks. Then, colitis was induced by supplementing 3% DSS in drinking water for 7 days, followed by 7 days of remission period to mimic ulcerative colitis symptoms. Goji berry supplementation ameliorated DSS-induced body weight loss, diminished diarrhea and gross bleeding, and resulted in a significantly decreased disease activity index, as well as DSS-associated colon shortening. Moreover, 30% mortality rate caused by DSS-induced colitis was avoided because of Goji berry supplementation. Histologically, Goji berry ameliorated colonic edema, mucosal damage and neutrophil infiltration into colonic intestinal tissue in response to DSS challenge, which was associated with decreased expression of chemokine (C-X-C motif) ligand 1 and monocyte chemoattractant protein-1, as well as inflammatory mediators interleukin-6 and cyclooxygenase-2. In conclusion, Goji supplementation confers protective effects against DSS-induced colitis, which is associated with decreased neutrophil infiltration and suppressed inflammation. Thus, dietary Goji is likely beneficial to inflammatory bowel disease patients as a complementary therapeutic strategy.
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Keywords: DSS; Goji berry; Gut; Epithelium; Inflammation; Inflammatory bowel disease

1. Introduction

Inflammatory bowel diseases (IBDs) encompass two major forms, ulcerative colitis (UC) and Crohn's disease (CD), which are chronic intestinal autoimmune diseases characterized by diarrhea, abdominal pain and rectal bleeding. IBD incidence has increased rapidly in the past few decades in both industrialized countries and many developing countries [1]. As a chronic disease, IBD consists of relapse and remission episodes. The major goal of therapeutic treatments is to reduce relapse and maintain the remission of symptoms [2]. These treatments include anti-inflammatory or immunosuppressive drugs, such as 5-amino-aslicylic acid, 6-mercaptopurine and antibiotics. However, side effects such as fever, cramps, diabetes and high blood pressure limit their long-term use [3]. Alternative strategies for IBD management without causing undesirable side effects are needed. Accumulating evidence suggests that certain dietary supplementations can confer protective effects against IBD [4]. For instance, grape seed extract improves epithelial structure and barrier function, and suppresses inflammation in immune-deficient interleukin (IL)-10 knock out mice to reduce the risk of IBD [5,6]. Dietary supplementation of black raspberry [7] and blueberry [8] exhibits anti-inflammatory effects on dextran sulfate sodium (DSS)-induced colitis. A clinical study with a large cohort of patients showed that long-term consumption of dietary fibers reduces the risk of IBD, among which fiber derived from fruits confers the strongest effect [9].

Goji berry, fruit of *Lycium barbarum*, is a traditional Chinese tonic food and exerts health-beneficial properties [10]. The *L. barbarum* polysaccharide (LBP) containing extract exhibits neuroprotective effects in different models of Alzheimer diseases, including beta-amyloid peptide neurotoxicity [11], homocysteine-induced neuronal damage and glutamate excitotoxicity [12], which are attributed to the reduced apoptosis and necrosis in cortical neurons. Recently, a milk-based Goji berry preparation named lacto-wolfberry, which contains 50% Goji berry and 25% skimmed milk, was shown to increase mice resistance to influenza infection through enhancing T-cell proliferation [13] and to exert protective roles in 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis in mice through anti-inflammatory effects [14]. However, its efficacies in different colitis models and in different colitis stages are unknown. Neither is there information on the

Abbreviations: CD, Crohn's disease; COX-2, cyclooxygenase-2; CXCL-1, chemokine (C-X-C motif) ligand 1; DAI, disease activity index; DSS, dextran sulfate sodium; IBD, inflammatory bowel disease; ICAM-1, intercellular adhesion molecule-1; IFN-γ, interferon gamma; IL, interleukin; LBP, *Lycium barbarum* polysaccharide; MCP-1, monocyte chemoattractant protein-1; MMP, matrix metallopeptidase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; qRT-PCR, quantitative reverse transcriptase PCR; TNBS, 2,4,6-trinitrobenzene sulfonic acid; TNF-α, tumor necrosis factor alpha; UC, ulcerative colitis.; VAAM-1, vascular cell adhesion molecule.

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beneficial effects of dietary Goji berry alone on intestinal inflammation, structure and overall health under inflammatory conditions.

DSS-induced colitis model is one of the most frequently used IBD models, which provides high uniformity and reproducibility of lesions mostly in the distal colon, mimicking human UC symptoms morphologically and pathophysiologically [15]. This model features weight loss, rectal bleeding and diarrhea associated with colon shortening, barrier dysfunction, inflammation and immune cell infiltration [16]. This study aims to investigate the protective effects of dietary Goji berry on IBD symptoms using a DSS-induced colitis mouse model.

2. Materials and methods

2.1. Animal care and experimental design

2.1.1. Mice and dietary treatments

C57BL/6 mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA) and bred in the Experimental Animal Laboratory Unit at Washington State University. All animal procedures were approved by the Washington State University Animal Care and Use Committee (ASAF#04316). Male mice were housed in a temperature-controlled room with 12:12-h light–dark cycle and fed a standard rodent diet (2018 Teklad Global 18% Protein Rodent Diet, Harlan Laboratory, Madison, WI, USA) and tap water *ad libitum* until dietary trial. At age of 6 weeks, 29 mice were randomly assigned to 2 groups receiving either a control diet (n=15, AIN-93G purified diet, Harlan Laboratory) or a diet supplemented with Goji berry (n=14, 1% of dry feed weight, USDA-certified organic Goji berry powder, Live Superfoods, Bend, OR, USA) for 4 weeks, when mice were challenged with 3% (w/v) DSS in water to induce colitis. Feed intake and body weight were monitored weekly during dietary treatments and DSS treatment.

2.1.2. Induction of colitis

Four weeks post dietary supplementation, mice in each dietary group were randomly divided into two subgroups receiving 0 (Con, n=5; Goji, n=5) or 3% (w/v) DSS (Millipore, Billerica, MA, USA) for 7 days to induce colitis (DSS-Con, n=10; DSS-Goji, n=9), followed by plain drinking water for 7 days to recover (Fig. S1). All mice were under their respective diets during DSS induction and recovery and had free access to water and food.

2.1.3. Assessment of symptoms and colitis score

The disease activity index (DAI) was calculated based on clinical symptoms of colitis including body weight, stool consistency, fecal bleeding and diarrhea, which were recorded daily [17].

2.2. Tissue collection

At necropsy, mice were anesthetized intraperitoneally with tribromoethanol (250 mg/kg body weight) followed by cervical dislocation. Blood was collected by cardiac puncture, and the resulting serum was stored at −80°C until assayed. The colon section was dissected, and the length from proximal to distal was further measured. A 5-mm segment of the distal colon was fixed in freshly prepared 4% (w/v) paraformaldehyde (pH 7.0), processed and embedded in paraffin. The remaining colon tissue was frozen in liquid nitrogen and stored at −80°C for further biochemical analyses.

2.3. Histological examination

Paraffin-embedded colonic gut tissues were sectioned at 5-μm thickness and subjected to hematoxylin–eosin (H&E) staining. Each colonic section was scored as previously described [18] with modifications. Briefly, the score system was based on three independent parameters: severity of inflammation (0–3), depth of injury (0–3) and crypt damage (0–4). The summation of these scores provides a total pathobiological score with 0 being a normal tissue and 10 being the most extensive/severe disease symptoms. Mice that died during DSS induction and recovery period were assigned a score of 10, which were excluded from any other analysis. Three sections per animal that covered the length of 600 μm of the distal colon were stained and evaluated in a blinded manner by two independent researchers.

2.4. Immunohistochemical staining of neutrophils

Immunohistochemical staining was conducted as previously described [19]. Briefly, colonic tissue sections were deparaffinized, rehydrated, antigen retrieved and blocked for 30 min with 1.5% goat normal serum and incubated with anti-Ly-6B.2 mAb (AbD Serotec, Raleigh, NC, USA) overnight at 4°C. Sections were then washed with phosphate-buffered saline with 0.05% Tween 20 and incubated with a biotinylated secondary antibody (Vector Laboratories, Burlingame, CA, USA) for 30 min. Signal was visualized using Vectastain ABC and DAB kits (Vector Laboratories). After counterstaining with hematoxylin, images were taken using Lecia DM2000 LED light microscope (200×, Leica Microsystems Inc., Chicago, IL, USA). Neutrophil infiltration was scored semiquantitatively from 0 (normal tissue) to 7 (intensive staining) based upon the depth (0–3) and extent of infiltration (0–4) [20,21] by two trained examiners in a blind manner. Three sections per animal were used for measurement, and for each section, a total of three to five areas encompassing >90% of the total mucosa were analyzed to determine the percent area positive as extent (0, none; 1, <25%; 2, 25%–50%; 3, 50%–75%; 4, >75%) and depth (0, none; 1, <1/3; 2, 1/3–2/3; 3, >2/3) of infiltration.

2.5. Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) analysis

The total RNA was extracted from colonic tissues by TRIzol Reagent (Sigma, St. Louis, MO, USA) followed by purification using RNeasy MiniRNA kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. cDNA was synthesized with the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA, UsA). qRT-PCR was conducted on a Bio-Rad CFX96 real-time thermocycler using β-tubulin as the housekeeping gene as previously described [22]. SYBR Green Master Mix (Bio-Rad) was used for all PCRs. Primers sequences were listed in Table S1.

2.6. Immunoblotting analyses

Immunoblotting analyses were conducted as previously described [19,23]. The band density of target protein was normalized to the β-tubulin. Antibodies against IL-6 and cyclooxygenase-2 (COX-2) were purchased from Cell Signaling Technology (Beverly, MA, USA). Anti-β-tubulin antibody was purchased from Sigma (St. Louis, MO, USA). Horseradish-peroxidase-conjugated anti-rabbit and anti-mouse secondary antibodies were purchased from Cell Signaling Technology.

2.7. Statistical analysis

Data were analyzed as a complete randomized design by GLM (2000); group means were expressed as mean ± S.E.M. A significant difference was considered as P value ≤.05.

3. Results

3.1. Dietary Goji berry treatment ameliorated disease activity in DSS-induced colitis

Goji berry supplementation had no effect on average daily feed intake and body weight gain (Fig. S1). DSS treatment resulted in dramatic body weight loss, watery and bloody diarrhea and even mortality in mice (Fig. 1); however, dietary Goji supplementation ameliorated body weight loss during DSS induction and recovery phase (Fig. 1A). Three mice in DSS-treated Con group died during the DSS induction, but no mice were lost in DSS-Goji group (Fig. 1B). Consistently, the severity of disease symptoms as indicated by DAI score was mitigated in Goji-berry-supplemented mice during DSS induction and recovery phase (Fig. 1C). As expected, without DSS induction, mice in both Con and Goji group showed no symptom (Fig. 1C). In line with improved body weight loss and DAI score, colon length shortening, an indirect marker of inflammation [24], was improved in DSS group supplemented with Goji berry compared with mice treated with DSS alone (Fig. 1D and E).

3.2. Dietary Goji berry alleviated DSS-induced mucosal damage

DSS treatment induced colonic structure damages, which were characterized by severe lesions throughout the mucosa, alteration of crypt structure, enhanced infiltration of immune cells into the mucosal and submucosal layer, and colonic edema (Fig. 2A). In mice supplemented with Goji berry, the histological damages induced by DSS treatment were substantially less (Fig. 2A), which were associated with reduced pathobiological score (Fig. 2B). Accompanied with damaged intestinal structure, DSS treatment resulted in increased pore-forming tight junction protein Claudin 2 and decreased barrier-forming tight junction protein ZO-2 (Fig. 3A and B), as well as up-regulated expression of adhesion molecules, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) (Fig. 3C). These alterations were numerically restored by Goji berry supplementation, although they were not statistically different (Fig. 3).

3.3. Dietary Goji berry suppressed inflammation in the colonic tissues

Based on immunohistochemical staining against Ly-6B, which recognizes neutrophils and a portion of activated macrophages [25], DSS treatment induced extensive and severe neutrophil infiltration in