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Dietary red raspberries attenuate dextran sulfate sodium-induced acute colitis☆

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Abstract

Persistent intestinal inflammation severely impairs intestinal integrity resulting in inflammatory bowel disease. Red raspberries (RB) are a rich source of bioactive compounds; their beneficial effect on the colitis protection was evaluated in the current study using a dextran sulfate sodium (DSS)-induced acute colitis mouse model. Six-week-old mice were fed a standard rodent research diet supplemented with RB (0 or 5% w/w, n=20 each group) for 6 weeks. At the 4th week of dietary treatment, approximately half of mice in each dietary group (n=12 each group) were subjected to 2.5% DSS induction for 6 days, followed by 6 days of recovery, to induce colitis. RB supplementation decreased body weight loss (P≤.01), disease activity index (P≤.01), and colon shortening (P≤.05) in DSS-treated mice. In addition, RB supplementation protected the colonic structure (P≤.01), associated with suppressed NF-κB signaling and reduced expression of inflammatory interleukin (IL)-1β, IL-6, IL-17, cyclooxegenase-2, and tumor necrosis factor-α in DSS-treated mice. RB supplementation reduced neutrophil infiltration, monocyte chemoattractant protein-1 mRNA expression, and xanthine oxidase content, but enhanced catalase content in DSS-treated mice. Consistently, RB supplementation reduced pore forming tight junction protein claudin-2, increased barrier strengthening claudin-3, zonula occluden-1 protein content and mucin (MUC)-2 mRNA level, and activated AMP-activated protein kinase (AMPK) in DSS-treated mice. In conclusion, dietary RB protected against inflammation and colitis symptoms induced by DSS, providing a promising dietary approach for the management of colitis.

Keywords: AMPK; DSS; Colitis; Inflammation; Red raspberry; Tight junctions

1. Introduction

Inflammatory bowel disease (IBD), encompassing Crohn's disease (CD) and ulcerative colitis (UC), is a chronic and relapsing disorder of the intestine. Around 1.5 million people are suffering from IBD in the United States, and is becoming common in the developing countries [1,2]. The common etiological factors of IBD include genetics, dysregulated immune response, chronic inflammation, unbalanced gut microbiota (dysbiosis), and defective mucosal barrier function [3]. Pathologically, colitis is characterized by prominent infiltration of neutrophils into colonic lesions, epithelial cell necrosis, and ulceration of the mucosal and submucosal layers [4]. Infiltrated neutrophils release reactive oxygen species (ROS) inducing local inflammation [5], and impairing barrier function [6]. The proper intestinal epithelial barrier function depends on the integrity of mucus layer, and the expression and assembly of tight junction (TJ) proteins. AMP-activated protein kinase (AMPK), the main regulator of cellular energy balance, is important for TJ assembly and proper barrier function of the gut epithelium [7,8]. Disrupted mucus layer and TJ complexes further exacerbate inflammatory response aggravating colitis [9,10].

Evident from the epidemiological studies, intake of diet high in fruits and vegetables, and low in fats has been associated with lower risk of IBD [11,12]. Berry fruits contain high amounts of polyphenolics [13,14], which are known for their anti-oxidative [15], anti-inflammatory [16], and anti-carcinogenic activities [17]. Red raspberries (RB) reduced oxidative stress in obese diabetic (db/db) mice [18], and its anthocyanin rich extract (RB-ARF) reduced inflammatory cascades in lipopolysaccharide (LPS)/IFN-γ-stimulated RAW264.7 macrophages [19]. The RB-ARF intraperitoneal injections suppressed histological indices of damage of colonic tissue in dextran sulfate sodium (DSS)-induced BALB/c mouse [19], a commonly used experimental colitis model resembling to human UC [20]. RB as a whole fruit contains substantial amount of health beneficial components including ellagitannins, flavonoids, vitamin C, fiber, and others [18,21], suggesting that whole RB supplementation might have a better protective effect than extracts [22] against DSS-induced colitis, which has not been tested yet. Therefore, we hypothesized that pre-feeding of whole RB in mice had a protective effect against the severity

Abbreviations: AMPK, AMP-activated protein kinase; COX-2, cyclooxegenase-2; DAI, disease activity index; DSS, dextran sulfate sodium; IBD, inflammatory bowel disease; IL, interleukin; NF-κB, nuclear factor kappa light-chain-enhancer of activated B cells; RB, red raspberries; ROS, reactive oxygen species; UC, ulcerative colitis; XO, xanthine oxidase; ZO-1, zonula occluden-1.

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of symptoms of DSS-induced experimental colitis possibly through its anti-inflammatory activity.

2. Materials and methods

2.1. Raspberry powder preparation and supplemental dose justification

Organic frozen RB (Lucerne Foods, Inc. Boise, ID, USA) were purchased from a local Safeway store in Pullman, WA. From the retail food label of frozen RB, the percent daily value (%DV) for total carbohydrates is 7%, dietary fiber is 20%, Vitamin C is 15%, calcium is 2%, and iron is 6%. RB were freeze-dried in VirTis freeze drier (Vertis, Gardiner, NY, USA), and were powdered using cyclone mill (Model 3010–060; UDY, Fort Collins, CO, USA). The total polyphenolics in RB is ~11 g gallic acid equivalent (GAE)/kg of dry weight. The RB powder contains 4.24±0.12% protein, 1.91±0.03% fat, 0.81±0.02% ash, 16.14±0.45% moisture. Per a recent publication, freeze-dried RB contains 35.1% dietary fiber in which 1.6±0.2% is soluble and 33.5±0.1% is insoluble fiber [18]. The powder was shipped overnight to the Research Diets, Inc. (New Brunswick, NJ, USA) for making customized rodent research diets containing, 0% and 5% of RB on dry weight basis. The dose of RB (5%) supplement was 50 g/kg of the diet. The average daily feed consumption of mouse was 2.40 g/mouse, which equals to 120 mg RB per day for an adult mouse of 20 g (i.e., 6 g RB/day/kg body mass). This converts to about 29.0 g of RB daily consumption for a 60 kg human per the published formula [23]. The similar supplementation level of RB was used in a recent mice study [18].

2.2. Experimental design and animal diet

Six-week-old wild-type C57BL/6J male mice (Jackson Laboratory, Bar Harbor, ME, USA) were randomized into 2 groups, receiving a standard rodent diet (CON, Research Diets Inc.), or a CON supplemented with RB (5% of dry feed weight, Research Diets Inc.) for 6 weeks. Detailed dietary information was listed in the Supplementary Table S1. Only male mice were used in the study to avoid the confounding effect of sex. There were 20 mice in each dietary group.

At 4th week of dietary treatment, mice at each dietary group were further randomly divided into two sub-groups, receiving a regular tap water with 0 or 2.5% DSS (MP Biomedicals, Santa Ana, CA, USA). This resulted in four dietary groups: neither DSS nor RB (CON, n=8), DSS without RB (DSSC, n=12), RB only (RB, n=8), and DSS plus RB (DSSRB, n=12). The CON and RB groups were given normal drinking water, while DSSC and DSSRB were given drinking water containing 2.5% DSS for 6 days, followed by 6 days of recovery period with normal drinking water. The DSS-induction and recovery period was used to mimic human UC symptoms [20,24]. Mice were monitored on daily basis for water consumption, body weight, fecal consistency, and blood in the stool throughout the DSS-treatment and recovery period. All mice were housed in a temperature-controlled room with a 12 h light and 12 h dark cycle and had free access to diet and drinking water. No difference was observed in the average amount of water consumption and feed intake among treatment groups. All animal procedures were approved by the Washington State University Animal Care and Use Committee (BAF#04316–010).

2.3. Assessment of colitis symptoms and disease activity index

The disease activity index (DAI) score was assessed by the combined score of weight loss compared to initial weight (scored as 0–4), stool consistency (scored as 0–4), and blood in the stool (scored as 0–4). The scores were recorded daily during the DSS- induction and recovery period, according to the previously described method [24].

2.4. Tissues collection and processing

Mice were anesthetized with CO_2 inhalation and followed by cervical dislocation. The colon section was dissected, and a 5 mm segment of distal colon was fixed in freshly prepared 4% (w/v) paraformaldehyde (pH 7.0), processed and embedded in paraffin. The remaining colon tissue was opened by a longitudinal cut, rinsed in PBS, frozen in liquid nitrogen, and stored at −80°C for later biochemical analyses.

2.5. Histological evaluation of colonic ulceration

Paraffin-embedded distal colonic tissues were sectioned at 5 μm thickness, deparaffinized and subjected to hematoxylin and eosin (H&E) staining. Histological examination and imaging were done under Lecia DM2000 LED light microscope (200x, Leica Microsystems, Chicago, IL, USA). For pathobiological scoring, each colonic section was scored blindly using a previously published score criteria [24], and 9 sections per animal at constant interval were used. The scores of crypt damage (0–4 scale), severity of inflammation (0–3 scale), and depth of injury (0–3 scale) were recorded individually. The summation of the scores resulted in the total pathobioloical score ranging from 0 to a maximum of 10 per distal colonic section.

2.6. Immunoblotting analyses

Immunoblotting analyses were conducted as previously described [25,26]. Briefly, protein extracts from colonic tissues were separated by sodium dodecyl sulfate polyacrylamide (10%) gradient gels and transferred to nitrocellulose membranes. After blocking with 5% w/v nonfat dried skimmed milk, membranes were overnight incubated with the selected primary antibodies at 4°C. Then the blot membranes were subsequently subjected to three times rinse of PBS with 0.5% Tween 20 (PBST), incubation with either IRDye 680 goat anti-mouse or IRDye 800CW goat anti-rabbit secondary antibodies (Li-Cor Biosciences, Lincoln, NE, USA) and then three times rinse of PBST. Finally, the bands were visualized and quantified using the Odyssey Infrared Imaging System and Image Studio Lite software (Li-Cor Biosciences, Lincoln, NE, USA). Bands density was normalized to the β-actin content. Antibodies against catalase, cyclooxegenase-2 (COX-2), interleukin (IL)-1β, IL-6, phospho-/total AMP-activated protein kinase (AMPK), and phospho−/total p65 were from Cell Signaling Technology (Beverly, MA, USA). Antibodies against claudin-2, claudin-3, occludin and zonula occluden-1 (ZO-1) were from Invitrogen (Rockford, IL, USA), and the antibody against xanthine oxidase (XO) was from Santa Cruz Biotechnology Inc. (Dallas, TX, USA). Anti-β-actin antibody was from the Developmental Studies Hybridoma Bank, University of Iowa (Iowa City, IA, USA).

2.7. Neutrophil immunohistochemical analyses

The immunohistochemical staining, scoring and analyses of neutrophil were carried out as described previously [26]. Briefly, paraffin-embedded colonic tissues sections were deparaffinized, hydrated and antigen retrieved. Sections were blocked in goat serum, overnight incubated with anti-Ly-6B.2 antibody (Bio-Rad Laboratories, Hercules, CA, USA), and then incubated with a biotinylated secondary antibody (Vector Laboratories, Burlingame, CA, USA) for 30 min. Signals were visualized using the Vectastain ABC and DAB peroxidase (HRP) substrate kits (Vector Laboratories, Burlingame, CA, USA) and hematoxylin counterstaining. Lecia DM2000 LED light microscope (200×, Leica Microsystems, Chicago, IL, USA) was used for images.

Neutrophil infiltration scores were assessed blindly by two researchers per our established method [27]. The scores for depth of neutrophil infiltration (scored as 0–3) and staining intensity (scored as 0–4), which was the percent area positive (0, none; 1, <25%; 2, 25–50%; 3, 50–75%; 4, >75%), were recorded individually. The summation of both scores result in 0–7 per distal colonic section. Nine sections per animal at constant interval were used for microscopic examination and score assessment.

2.8. Quantitative reverse transcriptase (qRT)-PCR analyses

Total RNA was extracted from the powdered colonic tissue using Dynabeads mRNA DIRECT™ Purification Kit (Invitrogen, Carlsbad, CA, USA) followed the manufacturer protocol. cDNA was synthesized with the iScript™ cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA, USA). qRT-PCR was performed on a Bio-Rad CFX384 real-time thermocycler. 18S was used as the reference gene [28]. Primer sequences used in the study were provided in Supplementary Table S2.

2.9. Statistical analysis

Data were analyzed as a complete randomized design using General Linear Model of Statistical Analysis System (2000), and were expressed as mean ± standard error of mean (S.E.M). Two-tailed Student's t test was used for calculating statistical significance between sample means. A significant difference was considered as P≤.05.

3. Results

3.1. RB supplementation ameliorates symptoms of DSS-induced colitis

Mice without DSS-treatment did not show any colitis symptom regardless of dietary groups (Fig. 1). A 6-day of 2.5% DSS-treatment successfully induced the clinical symptoms of colitis in mice (Fig. 1), as shown by body weight loss (Fig. 1A) and vast increase in DAI scores (Fig. 1B). All mice survived during the DSS-induction and recovery period. RB supplementation significantly reduced the body weight loss (Fig. 1A) and the DAI scores (Fig. 1B) during both the DSS-induction and recovery stages, indicating that RB had a protective role in both those stages. Moreover, an obvious swelling along with shortening in colon length was observed in DSS-treated mice, which was again ameliorated by RB supplementation (Fig. 1C, P≤.05).

3.2. RB supplementation protects histological architecture and reduces inflammation in DSS-induced colitis

The colonic tissues of mice without DSS-treatment showed a typical histological architecture as expected (Fig. 2A). The DSS-treatment caused injuries to the colon. Most of the epithelial cells were disappeared along with the loss of mucosa and crypts, indicating