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Preventive effects of cranberry products on experimental colitis induced by dextran sulphate sodium in mice

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ABSTRACT

With the prevalence of inflammatory bowel disease (IBD) and its associated risk for development of colorectal cancer, it is of great importance to prevent and treat IBD. However, due to the complexity of etiology and potentially serious adverse effects, treatment options for IBD are relatively limited. Thus, the purpose of this study was to identify a safe food-based approach for the prevention and treatment of IBD. In this study, we tested the effects of cranberry products on preventing dextran sulphate sodium-induced murine colitis. Our results suggest that both cranberry extract and dried cranberries-fed groups had a significantly reduced disease activity index, where dried cranberries were more effective in preventing colitis than cranberry extract. Shortening of colon length, colonic myeloperoxidase activity and production of pro-inflammatory cytokines were attenuated in animals fed dried cranberries compared to the controls. The current report suggests that cranberries can be applied to prevent and reduce the symptoms of IBD.

1. Introduction

Approximately 1.4 million Americans have inflammatory bowel disease (IBD), mainly Crohn's disease and ulcerative colitis, with the peak onset typically occurring in the second and third decades of life (Abraham & Cho, 2009). Patients with IBD have a significantly higher risk for the development of colorectal cancer (Askling et al., 2001), where colorectal cancer is the third most common cancer in both men and women in the United States (Siegel, Desantis, & Jemal, 2014). The symptoms of IBD include weight loss, diarrhea with blood or mucus, fever, and shortening of the colon. Crohn's disease is typically a transmural inflammatory disease, while ulcerative colitis is confined to the mucosa. Both Crohn's disease and ulcerative colitis show significant infiltration of immune cells, such as neutrophils, macrophages, T and B cells, and dendritic cells. Activation of these cells in the intestinal mucosa contribute to elevate local levels of pro-inflammatory cytokines, such as tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interferon-γ and interleukin-23 (IL-23) (Abraham & Cho, 2009; Baumgart & Carding, 2007; Baumgart & Sandborn, 2007; Lucas et al., 2007). These inflammatory cytokines are believed to play an important role in the pathogenesis of IBD.

There are a number of factors known to be linked to the incidence of IBD, such as genetic factors, environmental factors, geographic location, lifestyle differences, dietary factors, psychological factors, smoking, and history of gastrointestinal infection (Baumgart & Carding, 2007; Podolsky, 2002). Based on these, the treatment of IBD includes lifestyle alternations, and pharmacological and surgical treatments (Abraham & Cho, 2009). Recently, immune-suppressants, such as anti-TNF-α agents, are being used for treatment of IBD, although these treatments have significant and potentially serious side effects, thus limiting their usage over time (Abraham & Cho, 2009; Baumgart & Sandborn, 2007). Therefore, there still remains a great need for methods to prevent and maintain the proper immune and inflammatory responses in the long-term, without severe adverse effects for IBD (Podolsky, 2002; Rajendran & Kumar, 2010).

As potential significance, berries have been receiving great attention for their high content of antioxidant compounds and fibre to benefit human health. Among them, cranberries (*Vaccinum macrocarpon*), also called American cranberry, are rich in bioactive

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compounds and are known to have biological functions such as prevention of urinary tract infection and cardiovascular diseases (Blumberg et al., 2013). These cranberry bioactives may also have potential to alleviate inflammatory and immune responses (Murthy et al., 1993), including IBD. In fact, Popov et al. (2006) reported the benefits of cranberry fibre on acetic acid-induced colitis in mice. However, there is lack of knowledge on the benefits of other cranberry components, particularly phenolic compounds, in IBD. Thus the purpose of this study was to determine the role of cranberry bioactives, antioxidants and fibre, on IBD by using dextran sulphate sodium (DSS)-induced murine colitis as the experimental model. This is one of the most well-documented animal models for IBD and this model is similar to that of human ulcerative colitis in aspects of the progressions of inflammatory responses, clinical signs (diarrhea, occult blood, gross rectal bleeding), histopathological changes of the intestine including prominent sites, changes of intestinal microbiota and shortening of the colon (Chassaing, Aitken, Malleshappa, & Vijay-Kumar, 2014; Okayasu et al., 1990; Perse & Cerar, 2012).

2. Materials and methods

2.1. Materials

Dextran sulphate sodium (DSS) was purchased from MP biomedicals (MW: 36.000–50.000, MP Biomedicals, Solon, OH). Enzyme-linked immunosorbent assay (ELISA) kits for TNF-α and IL-1β were from R&D system (Minneapolis, MN, USA). Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase was purchased from Lucigen (Middleton, WI). dNTP Mix and Taqman® gene expression master mix were obtained from Applied Biosystem (Carlsbad, CA). Random oligonucleotide primers, and TNF-α, IL-1β oligonucleotide primers were purchased from Eurofins MWG Operon (Huntsville, AL). Primers for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were purchased from Biosearch Technologies (Petaluma, CA). 5-Aminosalicyclic acid (5-ASA) and hydrogen peroxide were purchased from Fisher Scientific (Pittsburg, PA). Hexadecyltrimethylammonium bromide and *o*-dianisidine dihydrochloride were from Sigma–Aldrich Co., (St. Louis, MO). Spray dried cranberry extract powder (HI-PAC 4.0), dried whole cranberry powder (PACRAN) and cranberry fibre were provided by Decas botanical (Carver, MA).

2.2. Methods

2.2.1. Animals and diets

Animal care and all procedures were approved by the Institutional Animal Care and Use Committee of the University of Massachusetts, Amherst. Sixty male BALB/c mice, aged 5–6 weeks old, were purchased from Jackson's Laboratory (Bar Harbor, ME) and were housed individually in wire-bottomed cages with a 12-h light–dark cycle. Animals were fed semi-purified AIN-93-based diet in powdered form from Harlan Laboratories (Madison, WI). The composition of the diet was as follows (ingredient, g/kg): corn starch, 465.7; sucrose, 255; cellulose 50; casein (vitamin free) 140; corn oil 40; mineral mix 35, vitamin mix 10; choline bitartrate 2.5; L-cystine 1.8. After a one-week adaptation period with the control diet, the animals were randomly divided into six experimental groups, with each group having 10 mice; Group 1, negative control, no DSS treatment; Group 2, DSS control; Group 3, 5-aminosalicylic acid (5-ASA), positive control; Group 4, 0.1% cranberry extract powder (HI-PAC 4.0, 0.011% phenolics and 0.04% cranberry fibre); Group 5, 1% cranberry extract powder (HI-PAC 4.0, 0.11% phenolics and 0.04% cranberry fibre); and Group 6, 1.5% dried whole cranberry powder (PACRAN, 0.011% phenolics and 0.7% cranberry fibre). The total phenolic content of the water extracts of cranberry products were determined by a method described previously (Dewanto, Wu, & Liu, 2002). The fibre content was based on data provided by Decas Botanical Synergies (Carver, MA).

Experiments were designed to achieve [1] two groups with the same cranberry fibre content but two different levels of total phenolics (Groups 4 & 5) and [2] two groups with the same total phenolic levels with different cranberry fibre content (Groups 5 & 6). Groups 1, 2, and 3 were fed the control diet throughout the experiment. Cranberry products were replaced at the expense of starch in the diet. Additional cranberry fibre was added to achieve the same amount of fibre in diets of Groups 4 and 5. Body weight and food intake were monitored daily during DSS treatment periods and weekly in other periods. Fresh foods were provided twice per week and food and water were provided *ad libitum*.

2.2.2. Induction and evaluation of severity of colitis

DSS solution (1 w/v%) was prepared in double distilled water and provided daily during the third and sixth weeks to induce experimental colitis, which are represented as the First and the Second DSS treatments in Fig. 1. 1% DSS is a relatively low concentration of DSS for inducing colitis in this model (Okayasu et al., 1990). The goal of this design was to observe any potential benefits of cranberry products, including the remission period. However, we did not observe any difference in the rate of recoveries between all groups (less severe colitis during the first DSS period resulted in a shorter recovery period compared to others). Thus, these data were not included in the manuscript. Water intake (data not shown) was monitored daily to ensure that each group consumed approximately equal amount of DSS-containing water during this time. The control group (Group 1) received double distilled water only. For the positive control, 5-aminosalicylic acid (5-ASA) (75 mg/kg/day) was dissolved in water only during DSS treatment periods in Group 3. The disease activity index (DAI) score, which combines scores for bloody stools, diarrhea and weight loss, is generally accepted as an important indicator of the severity of experimental colitis. Evaluation of the DAI was based on the method described by Cooper, Murthy, Shah, and Sedergran (1993) with slight modifications during the DSS treatment periods (3rd and 6th week). The method of scoring is shown in Table 1. Fecal blood, stool consistency and body weight were recorded daily during the DSS treatment period. The mean value of the combined scores of (i) fecal blood, (ii) stool consistency and (iii) body weight loss were used to calculate the DAI. A Hemoccult test kit (Beckman Coulter Inc., Pasadena, CA) was used to determine the occult blood in the fecal samples. The body weight loss was calculated as the percentage difference between the body weight prior to DSS treatment and the body weight on any particular day during DSS treatment (Cho et al., 2011).

At the end of the experimental period, animals were sacrificed by CO_2 asphyxiation after 4 h fasting. Blood samples were collected by cardiac puncture with Vaccutainer® (Greiner Bio-One North America Inc, NC) and then serum was separated by centrifugation at 3000 g for 20 min at 4 °C. These serum samples were used to determine TNF-α and IL-1β using enzyme-linked immunosorbent assay commercial kits (R&D system, Minneapolis, MN) as specified by the manufacturers. The entire colon was removed, gently washed with phosphate buffered saline, cleaned of fat and pat dried on filter paper. The colon length was measured between the colocecal junction and the anal verge.

2.2.3. The colon tissue analyses

Segments of the middle section of the colon were used for the extraction of total RNA using Trizol reagent (Invitrogen Corp., Carlsbad, CA). The total RNA was reverse-transcribed using M-MLV reverse transcriptase (Lucigen, Middleton, WI) and random primer (Eurofins MWG Operon, Huntsville, AL) and then mRNA levels of