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Research article

American ginseng attenuates azoxymethane/dextran sodium sulfate-induced colon carcinogenesis in mice

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ABSTRACT

Background: Colorectal cancer is a leading cause of cancer-related death, and inflammatory bowel disease is a risk factor for this malignancy. We previously reported colon cancer chemoprevention potential using American ginseng (AG) in a xenograft mice model. However, the nude mouse model is not a gut-specific colon carcinogenesis animal model.

Methods: In this study, an experimental colitis and colitis-associated colorectal carcinogenesis mouse model, chemically induced by azoxymethane/dextran sodium sulfate (DSS) was established and the effects of oral AG were evaluated. The contents of representative ginseng saponins in the extract were determined.

Results: AG significantly reduced experimental colitis measured by the disease activity index scores. This suppression of the experimental colitis was not only evident during DSS treatment, but also very obvious after the cessation of DSS, suggesting that the ginseng significantly promoted recovery from the colitis. Consistent with the anti-inflammation data, we showed that ginseng very significantly attenuated azoxymethane/DSS-induced colon carcinogenesis by reducing the colon tumor number and tumor load. The ginseng also effectively suppressed DSS-induced proinflammatory cytokines activation using an enzyme-linked immunosorbent assay array, in which 12 proinflammatory cytokine levels were assessed, and this effect was supported subsequently by real-time polymerase chain reaction data.

Conclusion: AG, as a candidate of botanical-based colon cancer chemoprevention, should be further investigated for its potential clinical utility.

1. Introduction

Colorectal cancer is one of the most common malignancies worldwide [1,2], and the 5-year survival rate is < 10% in the advanced stages [3]. Numerous effective drugs, including those currently used for cancer treatment, have been developed from botanical sources [4,5]. Thus, there still is a significant unexploited resource in herbal medicines.

In our previous studies, we assessed the colon cancer chemoprevention potential of American ginseng, a very commonly used

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herbal medicine in the USA. [6,7]. In an *in vivo* investigation, the tumor xenograft nude mice model was used and significant anti-tumor effects of ginseng compounds were observed [8]. However, the xenograft mice model was not a commonly appreciated model for colon cancer studies. In addition, the ginseng compound was administrated via intraperitoneal injection, an experimental approach, compared to the real world in which the route of administration of herbal medicines in humans is nearly always oral.

Inflammatory bowel disease is a group of chronic dysregulated inflammatory conditions in the large and small intestine of humans, and it is well known that chronic inflammation in the colon can lead to cancer [9–11]. An experimental colitis and colitis-associated colorectal carcinogenesis mouse model, chemically induced by azoxymethane (AOM)/dextran sodium sulfate (DSS), has been used often for colorectal cancer research [12,13]. AOM is a genotoxic colonic carcinogen frequently used to induce colon tumors [14,15].

We previously evaluated the effects of American ginseng (AG) in colorectal cancer chemoprevention in the AOM/DSS mouse model using a high-fat diet (20% fat) to mimic Western food [16]. In the present study, this established animal colon carcinogenesis model was used in mice fed with regular mouse chow (5% fat) reflecting an oriental diet, with or without AG supplement. To ensure the quality of the study botanical, high-performance liquid chromatography (HPLC) analysis was performed on the herb, and the contents of a number of important ginseng saponins were quantified. To extend previous tumor-related protein regulator observations, in this study, selected enzyme-linked immunosorbent assay (ELISA) for inflammatory cytokines and quantitative real-time polymerase chain reaction (qRT-PCR) were performed to elucidate the IBD related mechanisms of action.

2. Materials and methods

2.1. Chemicals and reagents

Standards of ginsenosides Rb1, Rb2, Rb3, Rc, Rd, Re, Rg1, Rg2, 20(*R*)-Rg2, Rg3, and Rh1 were obtained from Indofine Chemical Company (Somerville, NJ, USA) and Delta Information Center for Natural Organic Compounds (Xuancheng, AH, China). All standards were of biochemical-reagent grade and at least 95% pure. AOM was obtained from the NCI Chemical Carcinogen Reference Standard Repository, Midwest Research (Kansas City, MO, USA). DSS (molecular weight of 36–50 kDa) was obtained from MP Biomedicals (Solon, OH, USA). HPLC grade ethanol, *n*-butanol, acetonitrile, and dimethylsulfoxide were obtained from Fisher Scientific (Pittsburgh, PA, USA). Milli Q water was supplied by a water purification system (US Filter, Palm Desert, CA, USA). Hemoccult Sensa test strips were obtained from Beckman Coulter (Brea, CA, USA). Multi-Analyte ELISArray Kits for inflammatory cytokine analysis were obtained from Qiagen (Germantown, MD, USA).

2.2. Botanical materials and extract preparation

AG roots (4-year-old, *Panax quinquefolius* L.) were obtained from Roland Ginseng, LLC (Marathon, WI, USA). The voucher samples were authenticated by Dr Chong-Zhi Wang and deposited at the Tang Center for Herbal Medicine Research at the University of Chicago. AG extract was prepared with a slight modification from previous works [17–19]. The air-dried roots of AG were pulverized into powder and sieved through an 80 mesh screen. One kilogram of the powder placed into 12 L flask was extracted three times by heat-reflux with 8 L of 75% (v/v) ethanol at 95°C for 4 h each time. The extracting solution was filtered when hot. The gathered and combined filtrate was evaporated under vacuum with a Büchi Rotary Evaporator. The obtained extract was dissolved in 700 mL of water. The solution was extracted 3 times with 500 mL of water-saturated n-butanol. The mixed n-butanol phase was evaporated under vacuum and then lyophilized.

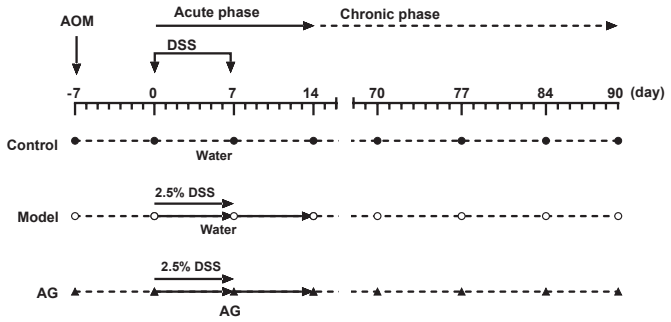

Fig. 1. Experimental protocol. Experimental A/J mice were divided into three groups, i.e., control (or negative control) group, model group, and American ginseng (AG) group. Animals in the model and AG groups initially received a single intraperitoneal injection of azoxymethane (AOM; 7.5 mg/kg). One week after the AOM administration (set as Day 1), mice in the model and AG groups received 2.5% dextran sodium sulfate (DSS) in drinking water for 8 consecutive days. Mice in the AG group also received oral AG extract 30 mg/kg/day for 90 consecutive days (Day 1–14 is the acute phase and then up to 90 days is the chronic phase).

2.3. HPLC analysis of herbal extract

Prior to pharmacological evaluation, the AG extract was analyzed using HPLC [20,21]. The HPLC system was a Waters Alliance 2960 instrument (Milford, MA, USA) with a quaternary pump, an automatic injector, a photodiode array detector (Model 996), and Waters Millennium 32 software for peak identification and integration. The separation was carried out on a Prodigy ODS(2) column (250 mm × 3.2 mm inner diameter) with a guard column (3.0 mm × 4.0 mm inner diameter) (Phenomenex, Torrance, CA, USA). For HPLC analysis, a 20-μL sample was injected into the column and eluted at room temperature with a constant flow rate of 1.0 mL/min. For the mobile phase, acetonitrile (solvent A) and water (solvent B) were used. Gradient elution started with 17.5% solvent A and 82.5% solvent B. Elution solvents were then changed to 21% A for 20 min, then to 26% A for 3 min and held for 19 min, at 36% A for 13 min, at 50% A for 9 min, at 95% A for 2 min, and held for 3 min. Lastly, eluting solvents were changed to 17.5% A for 3 min and held for 8 min. The detection wavelength was set at 202 nm. All sample solutions were filtered through a membrane filter (0.2 μm pore size). The content of the constituents were calculated using the standard curves of 13 ginsenosides. The measurement for the content analysis of the AG was performed in triplicate.

2.4. Animals and treatment protocols

The experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Chicago, Chicago, IL, USA. All experiments were carried out in male A/J mice, aged approximately 6 weeks, weighing 18–22 g, obtained from Jackson Laboratories (Bar Harbor, ME, USA). Mice were maintained under controlled room temperature, humidity, and light (12/12 h light/dark cycle) and allowed *ad libitum* access to standard mouse chow and tap water. The mice were allowed to acclimate to these conditions for at least 7 days prior to inclusion in the experiments.

As shown in Fig. 1, animals were separated into three groups ($n = 12$ per group): control (or negative control), model, and AG groups. All animals initially received a single intraperitoneal injection of AOM (7.5 mg/kg). One week after the AOM injection (set