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Oral administration of red ginseng powder fermented with probiotic alleviates the severity of dextran-sulfate sodium-induced colitis in a mouse model

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[ABSTRACT] Red ginseng is a well-known alternative medicine with anti-inflammatory activity. It exerts pharmacological effects through the transformation of saponin into metabolites by intestinal microbiota. Given that intestinal microflora vary among individuals, the pharmacological effects of red ginseng likely vary among individuals. In order to produce homogeneously effective red ginseng, we prepared probiotic-fermented red ginseng and evaluated its activity using a dextran sulfate sodium (DSS)-induced colitis model in mice. Initial analysis of intestinal damage indicated that the administration of probiotic-fermented red ginseng significantly decreased the severity of colitis, compared with the control and the activity was higher than that induced by oral administration of ginseng powder or probiotics only. Subsequent analysis of the levels of serum IL-6 and TNF-α, inflammatory biomarkers that are increased at the initiation stage of colitis, were significantly decreased in probiotic-fermented red ginseng-treated groups in comparison to the control group. The levels of inflammatory cytokines and mRNAs for inflammatory factors in colorectal tissues were also significantly decreased in probiotic-fermented red ginseng-treated groups. Collectively, oral administration of probiotic-fermented red ginseng reduced the severity of colitis in a mouse model, suggesting that it can be used as a uniformly effective red ginseng product.

KEY WORDS] Colitis; Fermentation; Inflammation; Probiotics; Red ginseng

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Introduction

Inflammatory bowel disease, such as Crohn's disease and ulcerative colitis, is a chronic and recurrent disease of the digestive system that occurs when a genetically vulnerable person is exposed to certain environmental factors or an inducer. It is characterized by an abnormal immune response to agents in the colorectal epithelia and environmental antigens including intestinal microflora [1-3]. From a histopathological point of view, colitis involves inflammation, ulcers, rectum length reduction, infiltration of inflammatory cells, increased neutrophil influx in intestinal tissues, and increased production of inflammatory cytokines [4-6]. Excessive secretion of cytokines induces a pathological condition and causes various inflammatory diseases, such as sepsis and organ damage [7]. Particularly in patients with ulcerative colitis, the level of tumor necrosis factor-α (TNF-α) significantly increases [8-9]. The terminal ileum and the inflamed parts of colon mucosa show increased levels of T cells and IL-6 [10-11]. Thus, regulation of inflammatory cytokines and mediators in the colon tissues will provide an

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important strategy for the treatment of colitis.

The intestinal canal is a main storage area for bacteria in the intestines and has a defense system comprising the intestinal barrier, secretion of immunoglobulins, and the phagocytic system [12]. The intestinal mucosa has the dual purpose of providing a barrier to prevent bacteria and toxins in the intestines from entering the blood stream, while simultaneously absorbing food ingredients. Probiotics are live microorganisms that pass through the intestinal canal and protect the surface of the mucous membrane from pathogens. Recently, there have been various studies with the hypothesis that probiotics are effective in protecting the intestinal mucosa and strengthening the intestinal barrier due to their cell permeability-moderating effect in chronic intestinal inflammatory diseases that are characterized by abnormal immune responses and increases in cytokine levels [13-14]. Moreover, probiotics have been shown to be effective in general health enhancement, by balancing bacterial flora in the intestines, improving metabolic diseases such as diabetes and obesity, and having immuno-modulatory effects [15-18].

The main pharmaceutical component of ginseng is saponin, which is also known as ginsenoside. However, ginseng also has other various non-saponin pharmacological components including acidic polysaccharides, polyacetylenes such as panaxydol, and organic compounds such as maltol. Parts of these components undergo chemical changes during the manufacturing process, generating saponin and other bioactive components [19]. The saponin in ginseng is mostly malonyl-ginsenoside and malonic acid is released from malonyl-ginsenoside, leaving trace saponins, including Rg3, Rg2, Rh2, Rs1, Rs2, and Rh4. Although red ginseng goes through the same pharmacological changes and is not entirely different from ginseng, there have been reports showing that the efficacy of red ginseng in enhancing blood circulation, cancer suppression, and defense against various infections is superior to that of ginseng [20-21]. Furthermore, the various efficacies of red ginseng including anti-inflammation have been reported to arise from Rg3, Rf, and Rh2 [22-23].

The saponin components of red ginseng are not directly absorbed into the body after administration, but first are converted to metabolites by intestinal microorganisms such as *Bifidobacterium* spp., *Lactobacillus* spp., and *Saccharomyces* spp. The metabolites exert the main pharmacological activities of red ginseng [24-25]. For this reason, despite the various and superior pharmacological effects of red ginseng, its efficacy after oral administration varies according to the intestinal conditions of an individual. Consequently, there is an assumption that the biological change in red ginseng in the anaerobic state is similar to that inside the intestine where intestinal microorganisms use fermentation to transform the components of red ginseng into final metabolites, which may help to produce a uniformly effective product that is easily absorbed, independent of the individual's intestinal conditions [26]. Therefore, we prepared the probiotic-fermented red ginseng powder and confirmed its efficacy in alleviating inflammation in a dextran-sulfate sodium (DSS)-induced mouse colitis model as known in red ginseng.

Materials and Methods

Preparation of probiotic-fermented ginseng powder and experimental materials

The red ginseng extract powder was obtained from Kunbo Inc. (Jinan, Korea). The red ginseng powder was suspended in water and fermented for 20 d with *Lactobacillus plantarum* (KFCC11611P) at 35−40 °C. Finally, the fermented red ginseng extract was freeze-dried and used in subsequent animal study. All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA), unless otherwise specified.

Qualitative analysis of the ginsenoside compositions

Qualitative analysis of the ginsenoside compositions in red ginseng powder was performed by high-performance liquid chromatography (HPLC). The HPLC system was equipped with an Agilent 1260 (Agilent Technologies, Inc., Santa Clara, CA, USA) with a DAD detector and an Agilent Eclipse plus C_{18} column (4.6 mm × 150 mm). The detection wavelength, flow rate, and column oven temperature were set at 203 nm, 1.6 mL·min^{-1}, and 30 °C, respectively. The solvent system for separation was consisted of purified water (solvent A) and acetonitrile (solvent B). The gradient program was as follows: 0 min, 18% of solvent B; 0−10 min, 18%−20%; 10−30 min, 20%−27%; 30−40 min, 27%−30%; 40−55 min, 30%−51%; 55−56 min, 51%−90%; 56−61 min, 90%; and equilibration with 18% of solvent B for 4 min. Sample injection volume was 10 μL. Ginsenoside compositions before and after the fermentation are compared in Table 1.

Table 1 Changes in ginsenoside compositions before and after the fermentation of red ginseng powder

Ginsenosides (mg·g^{-1})	Before fermentation	After fermentation
Ra1	7.978	4.172
Rb1	35.331	20.271
Rb2	16.392	13.484
Rb3	3.328	2.082
Rc	18.617	12.944
Rd	16.895	16.864
Re	0.651	2.636
Rf	3.170	2.672
Rg1	5.206	0.789
Rg2(s) Rh1(s)	3.185	4.677
Rg3(s)	3.341	10.445
Total concentration (mg·g^{-1})	134.460	91.037

Animals

Six-week-old female syngeneic BALB/c mice were purchased from Charles River Technology through Orient Bio, Inc. (Sungnam, Korea) and maintained under general specific