A Probiotic Preparation Duolac-Gold Ameliorates Dextran Sulphate Sodium-induced Mouse Colitis by Downregulating the Expression of IL-6

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Probiotics are live microorganisms that confer a health benefit on the host. Duolac-Gold is a mixture of seven probiotic bacteria containing three species of Bifidobacteria, two species of Lactobacillus, and Streptococcus thermophilus. The aim of this study was to assess the anti-inflammatory effects of Duolac-Gold in an inflammatory bowel disease (IBD) mouse model. IBD was induced by administering 1.5% dextran sulfate sodium (DSS) for 10 days. After induction of DSS-induced colitis, Duolac-Gold was orally administered at three different concentrations. Interestingly, Duolac-Gold treatment accelerated IBD healing, and anti-inflammatory activity was assessed by weight loss, length of the colon, and a microscopic damage score by histology. The expression of inflammatory related cytokines was measured in colon tissues and serum. Of these cytokines, the expression of interleukin-6 decreased remarkably after Duolac-Gold treatment. Taken together, these results suggest that Duolac-Gold treatment is effective in IBD healing by regulating IL-6.

Key words: Inflammatory bowel disease, Duolac-gold, probiotics, Cytokines, Interleukin-6

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory condition of the gastrointestinal tract with characteristic relapses and remissions, and mainly consists of ulcerative colitis (UC) and Crohn’s disease (CD) (1). While UC is limited to the colon, CD commonly affects the small intestine and/or the colon. The main symptoms of IBD are abdominal pain, fever, sweats, vomiting, diarrhea, rectal bleeding, and weight loss (2). Although the pathogenesis of IBD is not completely elucidated, it typically involves genetic susceptibility factors, immune-mediated injury, and a dysregulated immune response to host intestinal microflora (3).

Cytokines play a central role in the modulation of the intestinal immune system. They are produced by lymphocytes, monocytes, intestinal macrophages, granulocytes, epithelial cells, endothelial cells and fibroblasts. They have proinflammatory functions [interleukin (IL) -1, tumor necrosis factor (TNF), IL-6, IL-8, IL-12] or anti-inflammatory functions [IL-1 receptor antagonist (IL-1ra), IL-4, IL-10, IL-11, transforming growth factor (TGF) -beta]. Mucosal and systemic concentrations of many pro- and anti-inflammatory cytokines are elevated in IBD (4). Moreover, it is demonstrated that IL-6 plays a critical role in the development of Th1 cell-mediated IBD (5).

The human gut, the natural environment for a diverse microbial ecosystem, may be a major therapeutic target of research in biomedicine because gut microbiota are related to various diseases such as chronic inflammation, metabolic disorders, auto-immune diseases, and immune disorders (6,7). For example, normal microflora such as Lactobacillus and Bifidobacteria in the gastrointestinal tract are significantly reduced in patients with IBD, suggesting that IBD could be due to an imbalance of intestinal microflora (8,9). Therefore, normalizing the intestinal microflora may be an important means to treat IBD.

Probiotics have been used to improve symbiosis between enteric microbiota and the host or to restore states of dysbiosis (10). Probiotics have been defined as “living microorganisms in which ingestion in certain numbers exert health benefits beyond inherent general nutrition”, and they have several health beneficial effects including alleviation of lactose intolerance symptoms, reduction of cholesterol, alleviation of constipation, relief from vaginitis, and anti-cancer effects (11-14). Additionally, probiotic treatments are effec-
tive for inducing and maintaining the remission of IBD-induced colitis (15). However, the mechanisms are not fully understood.

Duolac-Gold is a formulation containing several species of *Bifidobacterium* and *Lactobacillus*. A recent study has reported that certain components of Duolac-Gold, particularly the Lactobacillus species, elicited inhibitory effects on the survival and growth of *Clostridium difficile*, a Gram-positive and toxin-releasing bacterium commonly responsible for various gastrointestinal disorders including colitis (16). Others have reported that *Bifidobacterium longum* ameliorated DSS-induced colitis by suppressing IL-17A (17). Furthermore, the Lactobacillus casei Shirota suppressed IL-6 in IBD have been documented (18). The combination of *Bifidobacterium* and *Lactobacillus* has also been reported to reduce the severity of IBD (19).

Many IBD animal models have been designed using various chemicals to study the pathogenic events, including dextran sulfate sodium (DSS), acetic acid, and 2, 4, 6-trinitrobenzenesulfonic acid (TNBS) (20). These models offer several advantages for developing preventive or therapeutic agents for the management of IBD. Of these chemical agents, the DSS-induced IBD mouse model provides an acute intestinal injury that mimics the intestinal status in patients with IBD (21). Moreover, the DSS-induced model provides human IBD-like symptoms and clinical histopathology compared to those of other chemically induced IBD models.

In this study, DSS-induced mouse colitis model was used to evaluate the protective effects of Duolac-Gold. In addition, mouse weight, length of colon, expression of cytokines, and the microscopic damage score were measured to assess the anti-inflammatory effects of Duolac-Gold.

**MATERIALS AND METHODS**

**Animals.** Eight-week-old black male C57BL/6 mice were purchased from Orient Bio (Seoul, Korea). The mice were randomly placed in cages in groups of five, and 10 mice were assigned to each group. During the 1 week adaptation period, the mice were induced to freely take pellet-type feeds and water under the conditions of 24 ± 2°C, a relative humidity of 40 ± 20%, and a 12 hr light cycle.

**Induction of IBD.** Chronic colitis was induced by administration of 1.5% DSS (MP Biomedical, Santa Ana, CA, USA) in drinking water for 10 days. Mouse health status was monitored daily throughout the experiment by weight loss, stool consistency, and perianal bleeding.

**Oral administration of Duolac-Gold.** Duolac-Gold is a probiotic formulation, including *Bifidobacterium laticis* (KCTC 11904BP), *Bifidobacterium longum* (KCTC 12200BP), *Bifidobacterium bifidum* (KCTC 12199BP), *Lactobacillus acidophilus* (KCTC 11906BP), *Lactobacillus rhamnosus* (KCTC 12202BP), and *Streptococcus thermophilus* (KCTC 11870BP). The Duolac-Gold was used freeze-dried as supplied by Cell Biotech Co. Ltd. (Gimpo-si, Gyeonggi-do, Korea). Animals were randomly assigned to five groups: positive control (PC), negative control (NC), Duolac-Gold (1 × 10⁸ CFU), Duolac-Gold (1 × 10⁹ CFU), and Duolac-Gold (1 × 10¹⁰ CFU). Duolac-Gold was orally administered to mice three times per week for 28 days.

**Reverse transcription-polymerase chain reaction (RT-PCR).** Total RNA was isolated from cells with a TRIzol reagent kit (Invitrogen, Carlsbad, CA, USA) and treated with DNase I, followed by RT-PCR amplification with an Access RT-PCR System Kit (Promega, Madison, WI, USA). PCR reactions were performed in a thermocycler (TaKaRa, Shiga, Japan) with the following cycling parameters: denaturation at 94°C for 5 min, 30 repetitive cycles of 94°C for 30 s, annealing at 55°C for 30 s, and elongation at 72°C for 30 s. The final extension was achieved at 72°C for 10 min. The primers used to amplify the cDNAs are shown in Table 1.

**Histopathology scoring.** Colon tissue was excised, fixed in 10% formalin for 24 hr, and washed with water. The tissue was dehydrated in alcohol (1 hr each in 70, 80, 90, and 100%) and xylene (three steps, 1 hr for each step) and embedded in paraffin. The paraffin block was sliced at a 7 μm thickness, stained with hematoxylin-eosin (H&E) (Sigma-Aldrich St. Louis, MO, USA) and periodic acid-Schiff (PAS), observed with a microscope, and quantified by histological scoring. Total severity was calculated by summing the scores for inflammation, glandular epithelial loss and erosion from proximal, middle and distal segments of colons (22).

**Mouse cytokine assay.** Blood samples were obtained by cardiac puncture from mice, and left at room temperature for 2 hr. The samples were centrifuged (4°C, 1500 xg, 15 min) to separate the serum. The samples were kept at 80°C until analysis. serum was thawed for the serum cytokine analysis, and the pro-inflammatory cytokine interleukin (IL)-6 (R&D Systems, Minneapolis, MN, USA) was con-

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**Table 1. Primer sequence for RT-PCR assays**

| Gene* | Sequence | Annealing temperature (°C) |
|-------|----------|---------------------------|
| IL-6  | Sence: 5'-ctctcagacaacctctggttc-3' | 56 |
|       | Antisence: 5'-ggacctctggttcac-3' | 56 |
| GAPDH | Sence: 5'-ggcagacactcagcatatc-3' | 56 |
|       | Antisence: 5'-ggatgaccagctgttgtg-3' | 56 |

*IL-6, interleukin 6, GAPDH, glyceraldehydes-3-phosphate dehydrogenase.
firmed through enzyme-linked immunosorbent assay using the i-Mark instrument (Bio-rad, Hercules, CA, USA).

**Statistical analysis.** Data are presented as mean ± standard deviation. Comparisons between groups were performed with one-way analysis of variance, followed by Student’s *t*-test and Tukey’s multiple comparison test. A *p*-value < 0.05 was considered significant.

**RESULTS AND DISCUSSION**

**Effects of Duolac-Gold treatment on body weight and colon length.** Despite the various therapeutic options for IBD, some patients are refractory to treatment or develop side effects (23). Therefore, a critical need exists for new treatment strategies. We investigated the effects of Duolac-Gold on IBD and a chronic colitis was induced by continuous administration of 1.5% DSS in drinking water (Fig. 1A). DSS-induced colitis produces many symptoms, including bloody diarrhea, weight loss, shortening of the colon, mucosal ulcerations, and neutrophilic infiltration into the left colon (23,24). We monitored weight loss, stool consistency, and perianal bleeding daily throughout the experiment. The DSS treatment caused weight loss and bloody diarrhea on day 10 (Fig. 1B). Then, Duolac-Gold was administered to three groups at different dosages (Fig. 1A). All Duolac-Gold-treated groups showed improved stool consistency compared with that in the PBS control group. Body weight also increased with Duolac-Gold treatment (Fig. 1B).

In a preliminary experiment, we found that DSS treatment caused significant shortening of the colon (data not shown). Thus, we measured colon length in all mice at the end of the experiment. All groups treated with Duolac-Gold treatment showed significantly increased colon length (Fig. 2A and Fig. 2B). This result suggests that Duolac-Gold treatment has the potential for relieving IBD symptoms.

**Microscopic examination of anti-inflammatory effects of Duolac-Gold.** The DSS treatment changed the histopathology and morphology in the colon in the IBD mouse model. All colon tissues were collected on day 36, and were paraffin-embedded and stained with H&E to visualize intestinal integrity (Fig. 3A). The untreated NC mice showed distinctive, non-disrupted and repeating crypt architecture

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**Fig. 1.** Duolac-Gold increases body weight of mice treated with DSS. (A) Experimental design and treatment protocol. (B) Effects of oral Duolac-Gold administration on weight change in the IBD mouse model. Animals were randomly assigned to three groups (PC, positive control; NC, negative control; DG, Duolac-Gold). Chronic colitis was induced by administration of 1.5% DSS. Duolac-Gold was orally administered 10 days later three times per week (black arrow; Duolac-Gold treatment).

**Fig. 2.** Duolac-Gold prevents DSS-induced shortening of colon length (A) Effects of Duolac-Gold on colon length in an IBD mouse model. Colon length in each group was measured at the end of the experiment. (B) Representative colon images in each group. Data are mean ± standard deviation; **p < 0.005 compared to NC group; ***p < 0.005 compared to PC group.
in the lamina propria (Fig. 3A, left panel). However, the 1.5% DSS treatment showed significant microscopic damage to the colon structure, including transmural inflammation with thickening of the muscularis, inflammatory cell infiltration, and significant loss of crypts in the colon, indicating severe clinical disease (Fig. 3A, middle panel). In contrast, mice treated with Duolac-Gold showed significantly reduced microscopic damage and restitution of nor-

![Fig. 3. Duolac-Gold administration decreases microscopic damage to colon tissue. (A) Colon tissue sections were stained with H & E and PAS. Representative histology from the proximal and distal regions of the colon from the NC, PC, and Duolac-Gold-treated animals. Scale bars = 50 µm (B) The total microscopic damage score was lower in the Duolac-Gold-treated group in each region of the colon. Data are mean ± standard deviation; ##p < 0.005 compared to NC group; **p < 0.005 compared to PC group.]

![Fig. 4. Duolac-Gold decreases pro-inflammatory cytokine IL-6 expression. (A) After mRNA isolation from colon tissues and cDNA synthesis, IL-6 was analyzed by RT-PCR, and normalized to GAPDH. (B) Levels of IL-6 expression in mouse serum. (C) Immunohistochemical analysis of IL-6 in colon. a. DSS treated mice colon, b. Duolac gold treated mice colon. Data are mean ± standard deviation; **p < 0.005 compared to PC group.]

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IL-1 production of various pro-inflammatory cytokines such as IL-6, a pro-inflammatory cytokine, plays important roles during infection and injury and is associated with persistent cytokine expression can result in rheumatoid arthritis, septic shock, and other chronic inflammatory diseases. However, excessive or persistent cytokine expression can result in rheumatoid arthritis, septic shock, and other chronic inflammatory diseases. IL-6, a pro-inflammatory cytokine, plays important roles during infection and injury and is associated with various diseases including IBD, diabetes, and cancer.

In a preliminary experiment, the DSS treatment induced production of various pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α in colon tissue of mice. In particular, IL-6 levels increased significantly by the DSS treatment compared with those in the PBS control mice, suggesting that elevated IL-6 expression lead to inflammation (data not shown). Therefore, to investigate whether IL-6 expression was altered during IBD healing induced by Duolac-Gold, the levels of IL-6 were assayed in colon tissues and serum. IL-6 expression decreased significantly in colon tissues following the Duolac-Gold treatment in comparison with mice that were not treated (Fig. 4A). In addition, IL-6 expression levels also decreased in serum and tissue following the Duolac-Gold treatment in comparison with mice that were not treated (Fig. 4B and Fig. 4C), suggesting that Duolac-Gold may be regulate IL-6 expression.

In conclusion, many studies have shown the positive effects of probiotics to treat stomach and intestinal diseases including IBD. Here, Duolac-Gold contributed to healing of DSS-induced colitis in an IBD mouse model. The anti-inflammatory effects of Duolac-Gold were measured by weight loss, colon length, and microscopic observations. The pro-inflammatory cytokine IL-6 decreased significantly in colon tissues and serum of mice following Duolac-Gold treatment. These results indicate that Duolac-Gold has anti-inflammatory effects by regulating IL-6 levels in a mouse model of IBD.

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