Soy Isoflavone Equol Perpetuates Dextran Sulfate Sodium-Induced Acute Colitis in Mice

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The effects of the soy isoflavones, genistein, daidzein and equol, on experimental colitis were examined. Equol severely perpetrated dextran sulfate sodium (DSS)-induced colitis as evaluated by the weight loss. Production of the anti-inflammatory cytokine, IL-10, from T cells was decreased in the equol-treated mice. The results show that the soy isoflavone, equol, played an important role in the inflammatory response in the gastrointestinal tract.

Soy foods have been consumed for centuries in Asian countries, and human clinical and epidemiological studies have revealed many potential benefits from the intake of soy products.1) For example, the consumption of soy foods may contribute to the lower incidence of coronary heart diseases,2) atherosclerosis3) and type 2 diabetes,4) and to the decreased risk of such types of carcinogenesis as breast and prostate cancer5,6) as well as better bone health and the relief of menopausal symptoms.7) Phytoestrogens, naturally occurring hormone-like compounds in plant food, have attracted considerable attention because of their antioxidative, antiatherogenic and anticarcinogenic potential. In addition of these properties, intense interest has been shown in the immune regulatory effects of soy isoflavones. The soy isoflavone, genistein, has been shown to suppress inflammatory response and allergic reactions.8,9) Chronic inflammatory bowel diseases (IBDs), mainly ulcerative colitis and Crohn’s disease, are inflammatory conditions of the digestive tract. Although the precise mechanism for the onset of IBD is unknown, clinical and experimental findings have suggested that IBDs are driven by a seemingly inappropriately elevated immune response to intestinal bacterial flora.10) Genistein and daidzein, the major soy isoflavones, have been extensively studied in the field of immunology. It has been shown that the soy isoflavone, equol, is a daidzein-derived metabolite and had unique properties compared to the properties of other isoflavones. It is possible that the effects of soy isoflavones on the immune function differ in human populations, because only 30–50% of humans can metabolize daidzein to equol.11) We examined in this study the effects of three soy isoflavones on acute dextran sulfate sodium (DSS)-induced colitis and investigated the mechanism of action of equol.

Female BALB/c mice (Japan SLC, Shizuoka, Japan) were maintained under specific pathogen-free conditions with a 12-h light:dark cycle at 25 ± 2 °C and 55 ± 10% relative humidity. The mice were provided with a soy isoflavone-free diet and water ad libitum. The composition of the diet was 20% casein, 44.7% α-starch (Oriental Yeast Co., Chiba, Japan), 22.3% sucrose (Mitsui Sugar Co., Osaka, Japan), 5% corn oil (Wako, Osaka, Japan), 2% cellulose, a 5% mineral mixture and 1% vitamin mixture (Oriental Yeast Co., Tokyo, Japan). Colitis was induced by feeding the mice with 4% DSS dissolved in drinking water which was provided ad libitum for 4 d. All experimental procedures were approved by the Animal Research Committee of the University of Tokushima.

Solutions of genistein, daidzein and equol (LC Lab., MA, USA) were freshly prepared daily in 25 mM Na2CO3. The mice were administered daily a 200-μL solution containing isoflavone from −7 to +5 d of DSS administration by gavage. Control mice were treated with 200 μL of 25 mM Na2CO3 alone instead of the isoflavone solution.

The mesenteric lymph node cells were stimulated with anti-mouse CD3 mAb for 48 h. The contents of IFN-γ, IL-4, IL-10 and TNF-α in the supernatant were separately quantified by using a mouse IFN-γ, IL-4, IL-10 or TNF-α ELISA kit (eBioscience, CA, USA) according to the manufacturer’s instructions.

Although immune modulation by soy isoflavones has been studied in an antigen (Ag)-immunized model,8) an allergic model9) and an autoimmune model,12) the effects of soy isoflavones on acute inflammatory colitis have not previously been examined. Mice that had been treated with genistein, daidzein or equol were given 4% DSS in this study. The mice were administered 20 mg of soy isoflavone/kg of body weight (BW). This dose was chosen on the basis of the results from previous studies.8,9,12-14) The body weight of the mice began to

Abbreviations: BW, body weight; DSS, dextran sulfate sodium; IBD, inflammatory bowel disease

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decrease from d4 of DSS administration. The mice treated with daidzein and especially those treated with equol showed a significant reduction in body weight. Compared to the control group, significant differences were observed from d4 to d7 in the equol-treated group and on d6 and d7 in the daidzein-treated group (Fig. 1A). Consistent with the results for weight change, the survival rate of the equol-treated group was significantly reduced; the respective survival rates of the control, genistein, daidzein and equol groups were 100%, 87%, 71% and 14%. The results showing that genistein did not affect the severity of colitis are unexpected, because genistein is an inhibitor of protein tyrosine kinase and is thought to act as an anti-inflammatory agent.15) We next examined the dose-dependent effect of equol on the severity of colitis. Mice were treated with 2, 10 or 20 mg of equol/kg of BW and then given the DSS solution. As shown in Fig. 2, the weight of the mice administered 20 mg of equol was significantly reduced, consistent with the results shown in Fig. 1, and the reduction in weight of the mice administered 2 or 10 mg of equol was not significantly different from that of the control mice. These results suggest that administering 20 mg of equol/kg of BW was necessary for the deterioration of DSS-induced experimental colitis. To examine the mechanism of action of equol, we focused on T cell subsets, because recent studies have shown that T helper (Th)-1 and Th17 subsets contributed to the development of DSS-induced experimental colitis.16,17) Cytokine production from mesenteric lymph node T cells was determined. Production of the anti-inflammatory cytokine, IL-10, in the equol-treated mice was significantly lower than that in the control mice. However, no significant difference was
apparent in the production of IFN-γ, IL-4 or TNF-α (Fig. 3). Furthermore, to examine the contribution of T cells to the equol-mediated immune modulation, T and B cell-deficient SCID mice were treated with equol and given the DSS solution. Although no significant difference in weight was found, the survival rate of the mice treated with equol was significantly lower than that of the control mice (data not shown). The results suggest that the perpetration of colitis by equol was caused by T cell-dependent and -independent mechanisms.

The soy isoflavone, equol, is known to have a unique property. The binding affinity of equol for estrogen receptors has been found to be similar to that of other isoflavones, but equol induced transcription more strongly than did any other isoflavone. The present study did not determine the target cells and mechanism by which equol perpetrated colitis. However, this study has shown for the first time that a soy isoflavone regulated the inflammatory response in the intestines. The fact that only 30–50% of people can produce equol suggests that the effect of a dietary soy isoflavone on the immune function might differ in equol-producers and non-producers. Since T cells and macrophages express estrogen receptors, estrogen receptor-mediated inflammatory modulation by equol is a possibility. An epidemiological study on the prevalence of allergic diseases and/or inflammatory bowel diseases in equol-producers and non-producers should provide an interesting clinical insight.

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