Efficacy of Wogonin in the Production of Immunoglobulins and Cytokines by Mesenteric Lymph Node Lymphocytes in Mouse Colitis Induced with Dextran Sulfate Sodium

Beong Ou Lim

Department of Applied Biochemistry, Konkuk University, Chungju, Chungbuk 380-701, Korea

Received June 18, 2004; Accepted September 7, 2004

We previously examined wogonin, isolated from Scutellaria baicalensis, chemical mediators, and IgE by mesenteric lymph node (MLN) lymphocytes in rats. The present study explores the effect of wogonin on the MLN lymphocyte function of mice given orally at 20 mg/kg for 2 weeks with dextran sulfate sodium (DS)-induced colitis. The results indicate that IgA levels in MLN lymphocytes were high, while IgE was low, in mice given wogonin compared to those given water. Also, fecal IgA concentration of DS in the wogonin group mice was significantly higher than in the DS group. Concentrations of interferon-γ and interleukin (IL)-2 of T cells by concanavalin A treatment was significantly higher in the wogonin fed group than in the normal group. Activation-induced IL-4, IL-5 and IL-10 secretion was lower in wogonin fed mice compared control mice after DS-induced colitis. For these reasons, we conclude that wogonin can alleviate the inflammation in DS-induced colitis brought about by an abnormal Th2 response.

Key words: wogonin; IgE and IgA; inflammatory bowel disease; cytokines; mesenteric lymph node

Inflammatory bowel disease (IBD) including Crohn’s disease (CD) and ulcerative colitis (UC) is a chronic, relapsing, and remitting condition of unknown origin that exhibits various features of immunological inflammation and affects at least in 1 in 1,000 people in western countries. There is an increasing amount of evidence that the immune system plays a critical role in the development and perpetuation of UC and CD. Studies in humans have implicated impaired mucosal barrier function, pronounced innate immunity, production of proinflammatory and immunoregulatory cytokines, and the activation of CD4+ T cells in the pathogenesis of IBD. There is clear evidence supporting a role for cytokine in the initiation and perpetuation of IBD. Recent studies have shown that the production of proinflammatory cytokines, tumor necrosis factor (TNF)-α, and interleukin (IL)-1, IL-6, and IL-8 at sites of inflammation is markedly enhanced in patients with UC and CD. Therapeutic trials of cytokine manipulation in such patients further support the idea that cytokines are important in the pathophysiology of these diseases.

Sulfasalazine (Sulf) has been mainly used for the treatment of UC, but the side effects remain a major clinical problem. Chinese herbal medicine has recently been of increased interest for the treatment of these disorders. Scutellaria radix has long been used as the Chinese medicine or as a medical plant in oriental countries for various purposes. It has been reported that Scutellaria baicalensis has diverse pharmacological effects. We reported that wogonin extracted from Scutellaria baicalensis has very potent antioxidative action in vivo as well as in vitro. But the study of the IBD of wogonin has not been totally appreciated. We therefore examined the effect of wogonin administration on IBD using mesenteric lymph node (MLN) lymphocytes isolated from Balb/c mice.

Many studies have shown that Chinese medicine has an important role for intestinal structure and function, but very few studies have focused on the effects of wogonin on chemotherapeutic-induced colitis. The purpose of this current study was to evaluate the intestinal immunoregulatory effects of wogonin on DS induced colitis in mice. Oral administration of DS in mice induces colitis resembling human UC. This model corresponds well to the clinical signs of IBD in humans and can serve as a reliable model for studies of this disease. In the present study, we focused on the response of MLN lymphocytes in mice after DS-induced colitis, and found a significant effect of wogonin on Ig, T cell and cytokine production as compared with the DS group.

Materials and Methods

Materials. 5,7-Dihydroxy-8-methoxyflavanone (wogonin) was purchased from Wako Co. (Osaka, Japan).
Concanavalin A, sulfasalazine and dextran sulfate sodium (DS) were purchased from Sigma (St. Louis, MO). Hemoglobin reagent (AM 503-K) was purchased from Asan Pharmaceutical Co. (Seoul, Korea). This compound was dissolved in phosphate buffered water (PBS, pH 7.4) or water and used for cell culture experiments. Monoclonal antibodies and cytokines were purchased from ID Labs Inc. (Amcelgrow, Ontario Canada). IgE related antibodies were purchased from Zymed Laboratories (San Francisco, CA). IgA related antibodies were purchased from Zymed Laboratories (San Francisco, CA). IgE related antibodies were purchased from Biosource International (Comarillo, CA). For the enzyme-linked immunosorbent assay (ELISA) of rat IgE and IgA, 0.05% Tween 20 in PBS (TPBS) for rinsing and Block Ace (Dainihon Pharmaceutical Co., Osaka, Japan) were used for blocking and dilution of antibodies as described previously. All other reagent grade chemicals were purchased from Sigma (St. Louis, MO).

Induction of colitis. Colitis was induced by means of drinking water supplemented with 5% DS (30,000 mol wt). This model was described in detail previously. Control mice were fed in a similar manner with drinking water containing no DS.

Study design and diets. Four-week old female Balb/c mice were obtained from Samtako Bio Korea (Osan, Korea). All animal-care techniques were performed within the guidelines approved by the Institutional Animal Care and Use Committee. The animals were maintained on a 12-h dark-light cycle and allowed free access to a nonpurified pellet diet and tap water under conditions of controlled temperature (25 ± 2 °C). They were adapted for 7 d prior to initiation of the experimental protocol. The mice were allowed an AIN-93G diet27) and drinking water ad libitum. All mice were divided into 4 groups of 7 mice. The mice were divided into 4 groups as follows: (1) Normal group: This group was fed the AIN-93G diet and drinking water without DS for 5 d. After 5 d, it was orally treated with water for 2 weeks only. (2) DS group: Acute colitis was induced by feeding mice for 5 d with 5% DS. After 5 d of DS, when the mice returned to drinking plain water, they were given wogonin orally at 20 mg/kg for 2 weeks. (3) Wogonin group: This group was fed the AIN-93G diet and drinking water without DS for 5 d. Wogonin was given orally at 20 mg/kg for 2 weeks. (4) DS + Wogonin (Wog) group: Acute colitis was induced by feeding mice for 5 d with 5% DS. After 5 d of DS, when the mice returned to drinking plain water, they were given wogonin orally at 20 mg/kg for 2 weeks. (5) DS + sulfasalazine (Sulf) group: Acute colitis was induced by feeding the mice for 5 days with 5% DS. After 5 d of DS, when the mice returned to drinking plain water, they were given Sulf orally at 50 mg/kg for 2 weeks.

After body weight and blood hemoglobin content were measured, the medical longitudinal length was measured.

Preparation of mesenteric lymph node (MLN). MLN was excised from Balb/c mice and lymphocytes were squeezed out into the RPMI 1640 medium (Invitrogen Corporation, Grand Island NY). After incubating the cells at 37 °C for 30 min to remove fibroblasts, 5 ml of the cell suspension was layered on 4 ml of Lympholytemic (Cedarlane, Hornby, Canada) and centrifuged at 1,500 × g for 30 min. The lymphocyte band at the interface was recovered and the cells were rinsed 3 times with the RPMI 1640 medium. The lymphocytes were cultured in a 10% FBS (Invitrogen Corporation, Grand Island NY)/RPMI 1640 medium, and the IgE and IgA content of the culture supernatant were measured by ELISA. Cell viability was measured by trypan blue staining. Cell viability by this preparation was more than 95% of the total cells.

Enzyme-linked immunosorbent assay of mice antibodies. Measurements of IgE and IgA were executed using sandwich ELISA methods, as reported previously by Lim et al.16,17)

Isolation of mesenteric lymph node (MLN) T-lymphocytes subsets. To the MLN lymphocytes suspended at 1 × 10^6 cells/ml, 10% FBS/PBS was added 5 ml of either CD4-FITC or CD8-PE monoclonal antibodies (Santa Cruz, CA), and incubated at 4 °C for 30 min. The stained lymphocytes were fixed by 2% paraformaldehyde and were counted by Epics Altrag™ flow cytometry (Beckman Coulter). Each analysis, including those of negative control samples, was based on at least 10^4 events after dead cells and gating on the basis of forward angle light scatter eliminated residual erythrocytes.24)

Fecal IgA measurement. Fecal pellets from each mouse in each group were collected from days 0 to 14, and stored at −80 °C. Feces samples were collected and prepared as described by Fukushima et al.29) In brief, fecal pellets were collected from each mouse into individual microcentrifuge tubes and suspended in protease inhibitor (0.1 mg/ml) 1 h at 4 °C. The pellets were then vortex homogenized and centrifuged at
15,000 g for 10 min, after which the supernatant fractions were collected and kept at −80 °C prior to IgA antibody measurement.

**Measurement of cytokines.** Supernatants from a 48 h ConA-activated MLN lymphocyte culture were obtained. Cytokines (IFN-γ, IL-2, TNF-α, IL-4, IL-5 and IL-10) were measured by ELISA using cytokine-specific capture and detection monoclonal antibodies as described previously.16,17,24,30

**Statistical analysis.** The statistical software program SPSS (SPSS for windows Ver. 10.0) was used for all data analyses. Standard SPSS statistical packages were used to estimate means, standard deviations, and one-way analysis of variance (ANOVA). Significant ANOVA effects were further analyzed by the Tukey method.

**Results**

**Growth variables**

The food intake and body weight are shown in Table 1A. The body weight of each mouse in the dextran sodium sulfate (DS) group and DS + Wogonin (Wog) groups was significantly lower (p < 0.05) than that of mice fed water or wogonin. The DS group had a lower food intake than the other groups. The difference between the normal and the DS group was significant, but was not significantly different for the wogonin, DS + Wogonin and DS + Sulf groups. As shown in Table 1B, the colon length of the DS-treated mice was significantly shorter than those of normal mice. The severity of ulcerative colitis-like lesions was most marked in the large intestine on the 5th day. The hemoglobin content of those treated with wogonin was significantly higher than that of the DS group.

**Fecal IgA concentration**

After feeding mice for 5 d for DS-induced acute colitis, the concentration of IgA in fecal extracts of mice was measured over a disease period of 14 d. The results are shown in Fig. 2. In the DS group IgA levels decreased for 4 day after DS-induced colitis, and slowly recovered thereafter. The peak level of the normal group after day 5 was between the wogonin and the DS group. From day 4 to day 11, the IgA level in the wogonin group was higher than that in the normal group. The normal and wogonin groups showed similar response patterns in which the IgA level remained unchanged until 5 d after DS-induced colitis. The IgA level of mice in the DS + Wog group reached a maximum level from day 6 to day 10, and then decreased. The IgA level of mice in the DS + Sulf group reached a maximum level from day 5 to day 8, and recovered thereafter.

**Mesenteric lymph node (MLN) Ig concentration**

In the absence of ConA, IgA concentration after 48 h incubation of MLN lymphocytes was higher in mice fed wogonin than in those fed water (Normal) (Fig. 3). It was significantly lower in the DS group than in the normal, wogonin and DS + wogonin groups. ConA treatment increased the concentration of IgA in cells from the water and wogonin-fed groups, and the value was markedly high in the former. The IgA concentration in MLN lymphocytes from the DS + Wog group was higher than in cells from the DS group. On the contrary, IgE concentration in MLN lymphocytes from mice DS was higher than in those fed DS + Wog when ConA was absent. When the cells were cultured with ConA, similar response patterns were observed.

**T cell population of MLN lymphocytes**

The proportion of CD4+ and CD8+ T cells in MLN lymphocytes was measured in relation to the changes in Ig concentration. (Table 2) There was a significant increase in the relative population of CD4+ T cells in the DS-induced colitis group compared to those fed water and wogonin. On the other hand, the proportion of CD8+ cells was higher in the normal group than in the wogonin group. However, the proportion of CD8+ T cells in the normal and DS + Wog groups remained unchanged. Consequently, the ratio of CD4+/CD8+ cells was lower in mice fed water than in the other groups. Also, the ratio of CD4+/CD8+ cells in the DS + Wog and DS + Sulf groups remained unchanged.

**Cytokine concentrations in MLN lymphocytes**

Various types of cytokine specifically regulate Ig
When lymphocytes were cultured for 48 h without ConA, the concentrations of IFN-\(\gamma\) and IL-2 were below the detection limit (data not shown). In the presence of ConA, the concentrations of IFN-\(\gamma\), IL-2 and TNF-\(\alpha\) were highest in cells from the wogonin group (Table 3A). As these results, the wogonin group with DS induced colitis showed higher production of IFN-\(\gamma\) and IL-2 than the normal or DS group. On the other hand, DS induced colitis was accompanied by a disturbance in IL-4, IL-5 and IL-10 (Table 3B). Activation with ConA significantly en-

### Table 2. MLN T Lymphocyte Populations in Normal and DS-Induced Colitis in Mice Treated with Wogonin

<table>
<thead>
<tr>
<th>Dietary Group</th>
<th>CD4(^+)</th>
<th>CD8(^+)</th>
<th>CD4(^+)/CD8(^+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>50.8 ± 0.5(^a)</td>
<td>12.3 ± 0.2(^d)</td>
<td>4.2 ± 0.1(^a)</td>
</tr>
<tr>
<td>DS</td>
<td>57.7 ± 0.2(^c)</td>
<td>10.6 ± 0.2(^e)</td>
<td>5.4 ± 0.1(^c)</td>
</tr>
<tr>
<td>Wogonin</td>
<td>53.0 ± 0.5(^b)</td>
<td>11.7 ± 0.2(^d)</td>
<td>4.5 ± 0.1(^b)</td>
</tr>
<tr>
<td>DS + Wog</td>
<td>59.3 ± 0.5(^d)</td>
<td>12.2 ± 0.2(^a)</td>
<td>4.9 ± 0.1(^c)</td>
</tr>
<tr>
<td>DS + Sulf</td>
<td>58.9 ± 0.4(^d)</td>
<td>11.7 ± 0.2(^b)</td>
<td>5.0 ± 0.1(^d)</td>
</tr>
</tbody>
</table>

Data are means ± SE of 7 mice. **Values without the same superscript letter are significantly different at \(p < 0.05\).**

---

**Fig. 2.** Levels of IgA in Fecal Extracts Prepared from Normal and DS-Induced Colitis Mice Fed on Wogonin.

Open diamonds: This group was fed the AIN-93G diet and drinking water for 2 weeks only. After 5 d, the mice were orally treated with water for 2 weeks only. Open triangles: Acute colitis was induced by feeding mice for 5 d with DS. After 5 d of DS, the mice were treated with water for 2 weeks only. Black squares: This group was fed the AIN-93G diet and drinking water without DS for 5 d. Wogonin was given orally for 2 weeks. Black circles: After 5 d of DS, the mice were given wogonin for 2 weeks. Black triangles: After 5 d of DS, the mice were given sulfasalazine for 2 weeks.

**Fig. 3.** IgA and IgE Production by Mesenteric Lymph Node Lymphocytes Isolated from Mice Fed on Wogonin.

MLN lymphocytes isolated from mice fed wogonin (2 x 10\(^6\) cells/ml) were cultured for 48 h in the absence (white bars) and presence (screened bars) of 25 mg/l of concanavalin A (ConA). The Ig contents of culture supernatants were measured by ELISA. The results are the means ± SE (\(n = 7\)). *Values not sharing a common letter are significantly different at \(p < 0.05\).*
wogonin inhibited the actions on IgE production by ConA-induced MLN lymphocytes. As shown in Fig. 2, mice fed wogonin in DS-induced colitis for 14 d showed significantly high levels of stool IgA compared to that of the DS-induced colitis group. In addition, IgA productivity during 48 h incubation of MLN lymphocytes was significantly higher in the DS + Wog group than in the DS group (Fig. 3). Since IgA plays a crucial role in the prevention of allergic reactions through interference with allergen absorption, this effect seems worthwhile. Increase of IgA concentration in clinically diseased animals might reflect an increase in immunoglobulin-mediated mucosa protection.

On the contrary, IgE productivity in the wogonin groups was significantly lower than in the normal group. In the case of DS-induced colitis similar response patterns were observed. These effects were not influenced by the presence of Con A in the culture media. In addition, wogonin enhanced IgA production and reduced IgE production by the lymphocytes in DSS-induced colitis. As a result, wogonin is expected to alleviate inflammatory reactions in the intestinal immune system.

T-helper cells can divided into subgroups with distinct regulatory and effector functions, based on their cytokine profiles. Th1 lymphocytes express IFN-γ, IL-2 and TNF-α, while Th2 lymphocytes are defined by the production of IL-4, IL-5 and IL-10. Th1 cells mediate delayed-types hypersensitivity, allograft rejection, and certain autoimmune diseases. Abnormal Th2 response is implicated in atopic disease. The cytokine profile of UC mucosa shows Th2 features, while CD is believed by some to be a Th1-mediated disease. The cytokines are important factors involved in inflammation and regulation of the immune response. IFN-γ, IL-2, TNF-α, IL-4, IL-5 and IL-10 are important in the initiation, regulation and perpetuation of inflammation in UC and CD.

In the present study, we found that the effect of wogonin on IFN-γ, IL-2 and TNF-α was strong, but IL-4, IL-5 and IL-10 concentrations were higher in mice with DS-induced colitis than in the normal group (Table 3). We found that increased Th1 cell cytokine and decreased Th2 cell cytokine can be reversed by feeding with wogonin even after the inflammation has become persistent. These data may suggest that treatment of DS-induced colitis with fed wogonin will strengthen the immune system in colitis by regulating cytokines such IL-4, IL-5 and IL-10, protecting against colitis-related damage. Therefore, the inhibitory effect of IL-4, IL-5 and IL-10 upon administration in DS-induced colitis might be mediated by the influence of IFN-γ, IL-2 and TNF-α by Th1 cells.

Our data clearly indicate wogonin mediated Ig production in the MLN lymphocytes (Figs. 2 and 3). Hence, it is possible that administration of wogonin regulates the immune response induced by helper T cells. Chinese medicine characteristically modified the proportion of CD4+ and CD8+ T cells in the MLN

### Table 3A. Activation-Induced Th1 Cytokine Secretion of MLN Lymphocytes in Dextran Sulfate Sodium-Induced Colitis in Mice

<table>
<thead>
<tr>
<th>Diet</th>
<th>ConA</th>
<th>IFN-γ (ng/ml)</th>
<th>IL-2 (ng/ml)</th>
<th>TNF-α (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td>+</td>
<td>664 ± 10*</td>
<td>86 ± 2*</td>
<td>287 ± 6*</td>
</tr>
<tr>
<td>DS</td>
<td>+</td>
<td>590 ± 9*</td>
<td>150 ± 3*</td>
<td>567 ± 12*</td>
</tr>
<tr>
<td>Wogonin</td>
<td>+</td>
<td>1136 ± 49*</td>
<td>193 ± 4*</td>
<td>218 ± 4*</td>
</tr>
<tr>
<td>DS + Wog</td>
<td>+</td>
<td>929 ± 9*</td>
<td>222 ± 6*</td>
<td>289 ± 6*</td>
</tr>
<tr>
<td>DS + Sulf</td>
<td>+</td>
<td>903 ± 7*</td>
<td>203 ± 5*</td>
<td>250 ± 10*</td>
</tr>
</tbody>
</table>

Data are means ± SE of 7 mice. *Values without the same superscript letter are significantly different at p < 0.05.

### Table 3B. Activation-Induced Th2 Cytokine Secretion of MLN Lymphocytes in Dextran Sulfate Sodium-Induced Colitis in Mice

<table>
<thead>
<tr>
<th>Diet</th>
<th>ConA</th>
<th>IL-4 (ng/ml)</th>
<th>IL-5 (ng/ml)</th>
<th>IL-10 (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td>+</td>
<td>62 ± 1*</td>
<td>79 ± 2*</td>
<td>56 ± 1*</td>
</tr>
<tr>
<td>DS</td>
<td>+</td>
<td>121 ± 4*</td>
<td>157 ± 5*</td>
<td>88 ± 2*</td>
</tr>
<tr>
<td>Wogonin</td>
<td>+</td>
<td>65 ± 1*</td>
<td>75 ± 3*</td>
<td>55 ± 3*</td>
</tr>
<tr>
<td>DS + Wog</td>
<td>+</td>
<td>50 ± 2*</td>
<td>87 ± 4*</td>
<td>56 ± 3*</td>
</tr>
<tr>
<td>DS + Sulf</td>
<td>+</td>
<td>56 ± 3*</td>
<td>82 ± 4*</td>
<td>58 ± 2*</td>
</tr>
</tbody>
</table>

Data are means ± SE of 7 mice. *Values without the same superscript letter are significantly different at p < 0.05.
lymphocytes. CD8⁺ T cells regulate CD4⁺ T cell development by producing IFN-γ or other regulatory cytokines that suppress the development of Th2 cells and may favor Th1 cell growth. Using T cell mitogen, ConA, administration of wogonin was found to mediate the immunoregulation indirectly through T cells. As shown in Table 3, there was a significant increase in the ratio of CD4⁺ to CD8⁺ T cells in the DS induced colitis group. The DS + Wog and DS + Sulf groups were similar in normalizing the CD4⁺ to CD8⁺ ratio, indicating the effect to be mediated by administration of wogonin. For this reason, the effect of wogonin is expected to be mediated, at least in part, through influence on the differentiation of T cells to become Th1 cells.

Considering the response of IgA and IgE, it is likely that wogonin can alleviate the inflammation by abnormal Th2 response in DS induced colitis. Although the exact mechanism by which wogonin modifies immune indices is not apparent at present, these observations might open a new aspect of the immunological role in inflammatory bowel disease of fed wogonin.

Acknowledgments

This work was supported by the Ministry of Science Technology through the Bio Food and Drug Research Center at Konkuk University and BioGreen 21 Program the rural development administration, Korea. The author deeply appreciate these support.

References


22) Rawsthorne, P., Shanahan, F., Cronin, N. C., Anton, P.


