Effects of Lactobacillus gasseri SBT2055 on Dextran Sulfate Sodium-Induced Ulcerative Colitis Model in Rats

Eiichi IMAI,¹ Kenji FUKUI,¹ Noriyasu OHTA,¹ Toshie TOMITSUKA,¹ Yasuyuki SETO,²* Shigeru FUJIWARA² and Hiroaki MASUNAGA¹

¹Research Institute of Life Science, Snow Brand Milk Products Co., Ltd., 519 Shimoishibashi, Ishibashi-machi, Shimotsuga-gun, Tochigi 329-0512, Japan
²Technology and Research Institute, Snow Brand Milk Products Co., Ltd., 1-1-2 Minamidai, Kawagoe, Saitama 350-1165, Japan

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The effects of a Lactobacillus gasseri SBT2055 (LG2055) on ulcerative colitis (UC) were studied in a rat model of UC induced by dextran sulfate sodium (DSS). Daily oral administration of LG2055 improved the characteristics of feces, body weight gain and food consumption. LG2055 treatment was also associated with a change of the spleen weight, and an improvement of the hematological parameters and histological findings of the large intestine. These findings indicate that LG2055 may be useful for the treatment and/or prevention of UC.

Key words: Lactobacillus gasseri; ulcerative colitis; probiotics; dextran sulfate sodium; rat

INTRODUCTION

Ulcerative colitis (UC) is an idiopathic inflammatory disorder, refractory to medical treatment, which is characterized by inflammation of the large intestine (19). Patients with UC often present bloody stools, diarrhea, abdominal pain, fever and body weight loss (19). Histopathologically, UC is characterized by inflammatory cells infiltration, erosion or ulceration, crypt abscess and cryptitis in the mucosa of the large intestine (14).

The etiology of UC is unknown and therefore no curative UC therapy has been established (5). At present, medical treatment, using mainly aminosalicylates and steroids, is commonly provided to patients with UC (5, 6). This therapy can improve symptoms; however, not all patients can be treated in this way because of lack of response to intensive pharmacotherapy, allergy to these drugs and severe adverse effects (6).

Diet is another UC therapy (5). A controlled diet is expected to reduce gastrointestinal stress caused by dietary factors such as fat and food residues (5). Moreover, this therapy produces little adverse effect (5). A dietary approach using lactic acid bacteria (LAB) is one of these therapies, and many clinical studies (9, 13, 20) have been already carried out using various kinds of LAB to examine their benefits for patients with UC.

Recently, we established a new LAB strain, Lactobacillus gasseri SBT2055 (LG2055) (4). L. gasseri is classified into the B1 subgroup, a predominant species in the human intestine, of L. acidophilus group (3). We found that LG2055 has unique in vivo antagonistic activities on Staphylococcus and it reduces the concentration of p-cresol in feces. In addition, it establishes in the intestinal tract of humans (4). Therefore, we tried to evaluate the preventive effects of LG2055 in a rat model of UC induced by dextran sulfate sodium (DSS), which is generally used for the study of therapeutic agents (6) and pathogenesis of UC (1, 11).

MATERIALS AND METHODS

Male CD (SD) IGS rats, six weeks old, were purchased from Charles River Japan Inc. (Kanagawa, Japan). The animals were housed under conditions of controlled temperature (23 ± 2°C), relative humidity (50 ± 10%), ventilation (15 times/hr), and light cycle (light: 07:00–19:00). Each animal was given a standard commercial diet (CRF-1, Oriental Yeast Industry, Co., Ltd., Tokyo, Japan) and tap water ad libitum. After a 1-week acclimation period, healthy animals showing good general condition were used for the study.

LG2055 (Snow Brand Milk Products Co., Ltd., Tokyo, Japan) was suspended in skim milk (10% w/v) and stored at less than −60°C until used. The mean concentration of viable LG2055 in the suspension was 1.2 × 10⁹ colony forming units (cfu)/ml. Dextran sulfate sodium (DSS, mean molecular weight 5000, total sulfur 15.0–20.0%) was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). DSS was dissolved in water and adjusted to a concentration of 3% (w/v).

*Corresponding author. Mailing address: Technology and Research Institute, Snow Brand Milk Products Co., Ltd., 1-1-2 Minamidai, Kawagoe, Saitama 350-1165, Japan. Phone: +81-492-42-8111. Fax: +81-492-42-8696. E-mail: ca2y-st@asahi-net.or.jp
Rats were allocated at random to the LG2055 group (n = 8) and the control group (n = 8). Rats in both groups were given drinking water containing 3% DSS ad libitum for 14 days according to the method of Kimura et al. (7). During that period, rats in the LG2055 group were given a suspension of LG2055 (1.0 × 10^{10} cfu/10 ml/kg) once a day for 14 days by gavage; while those in control group were given an equal volume of skim milk (10% w/v) also by gavage. Body weight, food and water consumption were measured every day.

Feces were examined once a day for appearance and evaluated according to the following scale: Score 0 = normal, Score 1 = bloody stools, Score 2 = bloody flux. On day 14, blood was sampled from the caudal vena cava under ether-induced anesthesia. The leukocytes count (WBC), erythrocytes count (RBC), hemoglobin concentration (Hb) and hematocrit (Ht) were determined using an auto-cell counter (E-4000, Sysmex Co., Ltd., Kobe, Japan). The spleen, cecum and colorectal region were excised and weighed. Representative animals exhibiting the typical clinical symptoms of UC were chosen from each group for the histological examination of the large intestine; their colorectum was cut at 1 cm from the anus, fixed in 10% buffered formalin and embedded in paraffin. Paraffin sections were cut at 4 μm-thickness from the posterior surface of each sample and stained with hematoxylin and eosin.

The data on the characteristics of feces were evaluated with the Mann-Whitney U test. The other data were evaluated with the Student’s t-test. P values less than 0.05 were considered statistically significant. In addition, for the analysis of food and water consumption, hematological findings and organs weight, data from untreated rats, which were obtained from a previous study performed under the same experimental conditions in our laboratory, were used as a reference.

RESULTS

In control rats, bloody stools and bloody flux were observed from day 6 and day 10, respectively (Fig. 1), and body weight gain and food consumption started to decrease around the same day when bloody flux was detected (Figs. 1 and 3, Table 1). As for the hematological examination, WBC was much higher than that of intact rats, while RBC, Ht and Hb were apparently lower than those of intact rats (Table 2). These changes in blood parameters indicated that rats with DSS-induced UC presented inflammation and anemia. It is also considered that the anemia was mainly reflecting the bloody stool and bloody flux, and the progress of inflammation and anemia might induce the loss of body weight gain. Histological examination of the large intestine revealed inflammation with erosion or ulceration in the control rats (Fig. 4). All these findings were consistent with those reported by Kimura et al. (7). Moreover, the weight of the spleen was increased in the control rats, probably due to extramedullary hematopoiesis induced in response to the anemia and colitis (Table 3). From these observations, the primary pathological event of this model of UC was considered to be the erosive or ulcerative inflammation associated with hemorrhage observed in the large intestine.

![Fig. 1. Fecal characteristics in dextran sulfate sodium-induced colitis rats in control group. Animals were treated orally with vehicle for 14 days. Each column represents the incidence rate of normal feces, bloody stool and bloody flux (Fecal samples were classified into any of these three categories).](image-url)
Fig. 2. Effect of *Lactobacillus gasseri* SBT2055 on the fecal characteristics in dextran sulfate sodium-induced colitis rats. Animals were treated orally with *L. gasseri* SBT2055 for 14 days. Each column represents the incidence rate of normal feces, bloody stool and bloody flux (Fecal samples were classified into any of these three categories).

* Significant difference from control group at \( p < 0.05 \).

Fig. 3. Effect of *Lactobacillus gasseri* SBT2055 on the body weight gain in dextran sulfate sodium-induced colitis rats. Animals were treated orally with *L. gasseri* SBT2055 (●) or vehicle (○) for 14 days. Data are the mean ± S.E.M. \((n = 8)\).

The feces of animals in the LG2055 group had a better appearance on day 13 as compared to that of the control group (Figs. 1 and 2); besides, the body weight gain and food consumption were also higher in the treated rats (Fig. 3 and Table 1). Furthermore, in the

Table 1. Effects of *Lactobacillus gasseri* SBT2055 on food consumption and water consumption in dextran sulfate sodium-induced colitis rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Food consumption (g/day)</th>
<th></th>
<th>Water consumption (ml/day)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 0–7</td>
<td>Day 7–10</td>
<td>Day 10–13</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>22.3 ± 0.4</td>
<td>21.3 ± 0.9</td>
<td>16.6 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>LG2055</td>
<td>8</td>
<td>23.6 ± 0.7</td>
<td>23.7 ± 0.9</td>
<td>21.6 ± 1.3*</td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>5</td>
<td>22.0 ± 0.9</td>
<td>22.7 ± 1.1</td>
<td>23.3 ± 1.2</td>
<td></td>
</tr>
</tbody>
</table>

Animals were treated orally with *L. gasseri* SBT2055 or vehicle for 14 days. Each value represents the mean ± S.E.M. * Significant difference from control group at \( p < 0.05 \).

LG2055 group, the weight of cecum and colorectum tended to be lower (Table 3), inflammatory changes in the large intestine were milder (Figs. 4 and 5), and the weight of the spleen was lower (Table 3).

**DISCUSSION**

There have been several reports that intestinal short-chain fatty acids (SCFAs) play an important role in the
Table 2. Effects of Lactobacillus gasseri SBT2055 on hematological findings in dextran sulfate sodium-induced colitis rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>WBC (x 10³/μl)</th>
<th>RBC (x 10⁴/μl)</th>
<th>Hb (g/dl)</th>
<th>Ht (%)</th>
<th>PLT (x 10⁴/μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controla</td>
<td>7</td>
<td>456 ± 46</td>
<td>398 ± 81</td>
<td>7.6 ± 1.6</td>
<td>24.1 ± 4.6</td>
<td>143.9 ± 7.5</td>
</tr>
<tr>
<td>LG2055</td>
<td>8</td>
<td>334 ± 32b</td>
<td>636 ± 53b</td>
<td>12.4 ± 1.1b</td>
<td>37.3 ± 3.1b</td>
<td>153.6 ± 10.4</td>
</tr>
<tr>
<td>Intact</td>
<td>5</td>
<td>94 ± 8</td>
<td>800 ± 11</td>
<td>15.8 ± 0.2</td>
<td>46.2 ± 0.6</td>
<td>132.4 ± 5.8</td>
</tr>
</tbody>
</table>

Animals were treated orally with L. gasseri SBT2055 or vehicle for 14 days. Each value represents the mean ± S.E.M.

a One animal died on day 14.

b Significant difference from control group at p < 0.05.

Fig. 4. Histological appearance of large intestinal mucosa from dextran sulfate sodium-induced colitis rat in control group. Animals were treated orally with vehicle for 14 days. Severe inflammatory cell infiltration into lamina propria (p) and submucosa (s), mixture of inflammatory exudation and necrotic mucosa representing erosion (e) and no mucosal epithelium are observed. The tissue was removed at 1 cm from the anus, fixed, embedded, sectioned, and stained with hematoxylin and eosin using standard procedures. Scale bars, 195 μm.

improvement of symptoms in DSS-induced UC models (2, 12) as well as in patients with UC (18, 21). Besides, SCFAs have been reported to serve not only as a metabolic substrate (15, 16) but also as a proliferating factor (8, 17) of colonocytes. Thus the positive correlation found in both the DSS model and patients with UC (2, 12, 18, 21) between the intestinal concentrations of SCFAs, especially of butyrate and attenuation of colitic lesions might be explained by these biologic actions of SCFAs.

We did not measure the intestinal concentrations of SCFAs in this study; however, there was a report that repeated oral administration of an antibiotics-resistant variant of LG2055 (LG2055SR) affected both the intestinal microflora composition and the metabolisms of several elements within the intestine, including intestinal concentrations of SCFAs (4). The concentrations of fecal acetic and butyric acids in particular tended to increase upon the ingestion of LG2055SR. This may explain the effectiveness of repeated oral administration of LG2055 on the symptoms of UC in the DSS-induced colitis model in this study. It is also well known that some kinds of probiotic intestinal LAB containing bifidobacteria produce SCFAs (10). It is possible that LG2055 ingestion affected the productivity of SCFAs by the intestinal microflora in the present study. However, the mechanism(s) involved need to be clarified in future studies.

In conclusion, repeated oral administration of LG2055 attenuated clinical and morphological changes in a rat model of UC. These results indicate that LG2055 might be useful for preventing UC and/or ameliorating the symptoms of the disease.

Table 3. Effects of Lactobacillus gasseri SBT2055 on organ weights in dextran sulfate sodium-induced colitis rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Spleen (g/100 g BW)</th>
<th>Colorectum (g/100 g BW)</th>
<th>Cecum (g/100 g BW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controla</td>
<td>7</td>
<td>0.345 ± 0.031</td>
<td>0.835 ± 0.052</td>
<td>0.583 ± 0.026</td>
</tr>
<tr>
<td>LG2055</td>
<td>8</td>
<td>0.251 ± 0.011b</td>
<td>0.741 ± 0.027</td>
<td>0.528 ± 0.021</td>
</tr>
<tr>
<td>Intact</td>
<td>5</td>
<td>0.205 ± 0.016c</td>
<td>NEc</td>
<td>NEc</td>
</tr>
</tbody>
</table>

Animals were treated orally with L. gasseri SBT2055 or vehicle for 14 days. Each value represents the mean ± S.E.M.

a One animal died on day 14.

b Significant difference from control group at p < 0.05.

c Not examined.
Fig. 5. Histological appearance of large intestinal mucosa from dextran sulfate sodium-induced colitis rat in *Lactobacillus gasseri* SBT2055 group. Animals were treated orally with *L. gasseri* SBT2055 for 14 days. Decreased cellularity of inflammatory cells in lamina propria (p) and submucosa (s) and nearly intact mucosa covered with epithelial cells (arrowhead) in comparison with control rat in Fig. 3 can be seen. The tissue was removed at 1 cm from the anus, fixed, embedded, sectioned, and stained with hematoxylin and eosin using standard procedures. Scale bars, 195 µm.

REFERENCES


