Germinated Barley Foodstuff Improves Constipation Induced by Loperamide in Rats

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The effects of germinated barley foodstuff (GBF) derived from the aleurone and scutellum fractions of germinated barley low-lignified hemicellulose were examined in Sprague-Dawley rats with constipation induced by loperamide by addition to the diet (2 mg/kg body weight). Bowel movements were higher in the GBF-fed rats than in the cellulose-fed rats used as a control. Fecal water content was also higher in the GBF-fed rats. The concentration of short chain fatty acids in cecal content, especially butyrate, was significantly higher in the GBF-fed rats than in the cellulose-fed rats. These findings suggested that GBF helps normalize defecation not only in diarrhea but also constipation.

Key words: germinated barley; butyrate; constipation; dietary fiber; rat

Dietary fiber may be classified into two groups, water-soluble and water-insoluble fibers. The water-soluble fibers such as polydextrose, facilitate defecation by increasing osmotic pressure in the bowel lumen, resulting in softening of feces, while the water-insoluble fibers, such as wheat bran and cellulose, promote defecation by increasing fecal volume in the bowel. However, water-insoluble dietary fibers may cause mucosal damage due to mechanical stimulation of the intestinal mucosa, especially in patients with inflammatory bowel disease.

Germinated barley foodstuff (GBF) is a novel foodstuff derived from the aleurone layer, scutellum, and germ of germinated barley. GBF mainly consists of water-insoluble dietary fibers and glutamine-rich proteins, and increases 1) the fecal volume and its water content, 2) the protein, DNA, and RNA levels in jejunal mucosa, and 3) the production of short chain fatty acids (SCFA), particularly butyrate, in rats. Furthermore, GBF can prevent the diarrhea induced by water-soluble dietary fibers and severe diarrhea in experimental colitis induced by dextran sulfate sodium, in rats.

Some oligosaccharides are fermentable and improve the intestinal environment, probably through the production of SCFA by microflora. Dietary fiber has been reported to be effective against not only diarrhea but also constipation through the production of bacterial SCFA. In this study, we examined the preventive effect of GBF on experimental constipation induced by loperamide in rats.

The method of production and chemical composition of GBF were described in our previous studies. Cellulose (CE) was added to the diet as the control (CE diet). To the test diet, 10% GBF was added, the protein and dietary fiber contents being adjusted to the same levels as those in the CE diet (14.8% protein and 3.0% dietary fiber) by changing the levels of casein and cellulose. Detailed dietary compositions are shown in Table, and the chemical composition of GBF has been described previously.

Experimental constipation was induced by oral administration of loperamide. The amount of loperamide (loperamide hydrochloride, Sigma Chemical Co., St. Louis) in the diet was adjusted to give 5 mg/kg body weight/day for each rat. All the dietary components were purchased from Oriental Yeast Co. (Tokyo, Japan), and all other experimental reagents from Wako Pure Chemical Co. (Osaka, Japan).

### Table Composition of the Experimental Diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>CE</th>
<th>GBF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(g/kg of diet)</td>
<td>(g/kg of diet)</td>
</tr>
<tr>
<td>Casein</td>
<td>146</td>
<td>100</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>35.0</td>
<td>35.0</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>30.0</td>
<td>GBF</td>
</tr>
<tr>
<td>GBF</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>Loperamide</td>
<td>(0.1)</td>
<td>(0.1)</td>
</tr>
<tr>
<td>Corn oil</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>727</td>
<td>703</td>
</tr>
</tbody>
</table>

1 The vitamin mixture was prepared according to the AIN 93 vitamin mixture protocol.
2 The mineral mixture was prepared according to the AIN 93G mineral mixture protocol.
3 Volume of loperamide was estimated as below: (0.20 kg body weight × 10 mg loperamide/kg body weight)/0.02 kg food intake = 450 mg as loperamide/kg diet.
4 CE, cellulose; GBF, germinated barley foodstuff. The protein and neutral detergent fiber contents in both diets were adjusted to the same levels.

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Male Sprague-Dawley rats each weighing about 50 g were purchased from Charles River Japan Co. (Yokohama, Japan). They were individually housed in metabolic cages in a room kept at 22±1°C, with lighting from 8:00 a.m. to 8:00 p.m. daily. The rats were allowed free access to the respective diets and drinking water. The animals were first fed on a laboratory diet (Nihon Clea Co., Tokyo, Japan) for 7 days during the acclimatization period. They were then divided into 2 groups (n=10), each group being fed either the CE- or GBF-containing diet for 10 days. On day 10, loperamide was added to both diets, and the rats were subsequently fed the respective diets for another 4 days. During the experimental period, the food intake and body weight were measured every 3 days. Feces excreted during the final 3 days (on days 12, 13 and 14) of the loperamide administration period were collected after the number of feces had been counted, and stored at −80°C. After lyophilization, dry weight of the feces was measured. On day 14, the rats were anesthetized with urethane, and the large intestine and the cecum were isolated. The feces in the rectum were collected and fecal water content was measured by lyophilization as described previously. The concentration of SCFA in cecal content was measured by gas-liquid chromatography.

Statistical comparisons between the two groups were made by using Student’s t-test. In all statistical analyses, an associated probability (p value) of <5% was considered as significant.

Figure (A) shows the body weight, (B) daily food intake during the experimental period, (C) fecal dry weight, (D) number of feces (on days 12, 13 and 14), (E) fecal water content, and (F) cecal SCFA concentration on day 14.

There were no significant differences in body weight and food intake between the CE and the GBF groups during the experimental period. However, food intake in the two groups decreased slightly, immediately after the initiation of loperamide administration (day 10). This decrease in food intake was reflected in slight retardation of body weight gain.

Fecal dry weight and number of feces in the GBF group were significantly higher than those in the CE group. In normal rats, fecal dry weight in the CE and GBF groups was 2.89±0.09 and 3.30±0.07, respectively, but in this constipation model, the weight was 1.07±0.09, and 1.89±0.09, respectively. The decrease in the fecal dry weight by constipation in the CE group was 37% (1.07/2.89), but that in the GBF group was 57% (1.89/3.30). Thus, GBF not only increased the fecal dry weight, but also prevented the retardation of fecal excretion in the experimental constipation model, more than CE. A similar tendency was observed in the number of feces.

The water content of feces in the rectum was also significantly higher in the GBF group than in the CE group. GBF is considered to increase not only the fecal volume but also water content. In our previous study, GBF feeding increased the fecal water content in normal rats. That is, GBF improved defecation not only in constipation, but also in normal rats.

**Fig.** Change in Body Weight (A) and Food Intake (B) of Rats during the Experimental Period.

Fecal dry weight (C), number of feces (D) during the last 3 days. Fecal water content (E) and short chain fatty acid concentration in cecal contents (F) of rats on day 14. The rats were fed on the diet containing loperamide (day 10-14) together with cellulose (CE), or germinated barley foodstuff (GBF) throughout the experimental period.

*p<0.05 compared with the CE group. For statistical analysis, Student’s t-test was used.

GBF increased cecal acetate and butyrate contents, but had no significant effect on the propionate content. GBF is considered to be used efficiently by microflora during constipation, since the cecal content of short chain fatty acids was significantly higher in the GBF group than in CE group.

Previously, we demonstrated the anti-diarrhea effect of GBF in the acute diarrhea model induced by water-soluble dietary fiber. Furthermore, GBF had a potent water holding capacity in the small intestine in cecocolectomized rats. In this study, GBF promoted defecation in rats with constipation induced by loperamide, probably by adjusting the fecal water content at an appropriate level. Loperamide is known to inhibit the intestinal fluid secretion and affect the cyclic AMP level or adenylyl cyclase activity in epithelial cells. Loperamide may stop diarrhea by disturbance of intestinal motility, that is, by promoting constipation. In this experiment, much indigestible content was observed in the small intestine.

Oligosaccharides and psyllium have been reported to help normalize defecation in both constipation and diarrhea. This effect was considered to be caused by the production of endogenous (bacterial) short chain fatty acids. In this study, a significantly larger amount of short chain fatty acids, especially acetate and butyrate, was produced in the cecum in the GBF group than in the CE group.

In addition to the adequate fecal water content, the production of short chain fatty acids in the cecum may
be involved in the promotion of defecation including bowel movement and fecal output. The detailed action mechanism in the constipation model must be studied.

References