Note

Antidiabetic Effect of Lactobacillus GG in Streptozotocin-induced Diabetic Rats

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Neonatally streptozotocin-induced diabetic (n-STZ) rats were given food containing Lactobacillus GG cells (GG) or a control diet (control), from 9 to 18 weeks of age. The GG cells significantly lowered the blood hemoglobin A1C (HbA1C) level and improved glucose tolerance in n-STZ rats (p < 0.05). In the GG group, the serum insulin level at 30 min after glucose loading was significantly higher than in the control group (p < 0.05).

Key words: Lactobacillus GG; antidiabetic effect; glucose tolerance; HbA1C; n-STZ rats

Intestinal flora has been shown to influence human health in many studies.1) The best control of the flora balance in the intestine is achieved by the intake of lactic acid bacteria, which is thought to be effective in lifestyle-related diseases such as hypertension, cholesterolemia, diabetes, and cancer.1) There have been many studies on the effects of lactic acid bacteria on hypertension,2) cholesterolemia,3) and cancer.4) However, their antidiabetic effects have hardly been studied.

Lactobacillus strain GG is isolated from the feces of healthy humans, and has been shown to survive passage through the human gastrointestinal tract.5) L. GG is one of the most thoroughly studied probiotics, and has beneficial effects such as prevention of diarrhea, antitumor activity, immune system stimulation,6) and hypocholesterolemia.7) We hypothesized that L. GG, because of its beneficial effect, might also induce antidiabetic activity. Thus, in this study, we investigated the effects of ingesting GG cells on blood glucose levels and glucose tolerance in neonatally streptozotocin-induced diabetic (n-STZ) rats.8)

The L. GG used in this study was obtained from Takanashi Milk Products Co., Ltd. (Yokohama, Japan). It was inoculated into MRS broth (Oxoid Ltd., England) and incubated at 37°C for 18 h. The whole cells were harvested by centrifugation at 2000 × g for 20 min and washed twice with sterile distilled water. The washed cells were lyophilized, and kept at −80°C.

The animals were maintained in accordance with the guidelines of governmental legislation in Japan (1980), and housed in an air-conditioned room at 23 ± 1°C with 55 ± 5% humidity on a cycle of 12 h light and dark. Wistar pregnant rats were obtained from CLEA Japan Inc. (Tokyo, Japan). After confinement, diabetes was induced in 2-day-old male neonates by administration of streptozotocin (60 mg/kg B.W.i.p.), while normal rats received 0.9% saline alone. The diabetic and normal rats were weaned at 4 weeks after birth and fed commercial pelleted chow (CE-2, CLEA Japan) until 8 weeks of age. The diabetic rats were divided on the basis of glucose tolerance into two groups of 6 rats; one was given powdered CE-2 chow alone (Control) and the other powdered CE-2 chow plus 2% lyophilized GG cells (GG). The 6 normal rats were given the powdered CE-2 chow alone (Normal). Each group was given 20 g of their respective diets daily for 9 weeks. Water was supplied ad libitum.

Before (8-weeks-old) and after administration of the experimental diets (18-weeks-old), a glucose tolerance test was done on 18-h fasted rats. Blood samples were collected from the tail vein 0, 15, 30, 60, and 120 min after oral administration of 1 g/kg B.W. glucose, and glucose levels were measured by the glucose oxidase method (Glutest Ace, GT-1640, Sanwa Kagaku Kenkyusho, Nagoya, Japan). The blood samples were centrifuged at 2000×g for 10 min, and the serum obtained was analyzed for insulin 0 and 30 min after glucose loading, using an enzyme immunoassay kit (Morinaga Seikagaku Kenkyusho, Yokohama, Japan). At the end of the experimental period, blood samples were collected from the tail vein after 6-h without food. The blood glucose levels were measured by the method noted above, and hemoglobin A1C (HbA1C) levels were measured by the DCA-2000 system (Bio Medical, 1 To whom correspondence should be addressed. Tel: +81-238-22-7396; Fax: +81-238-22-7333; E-mail: tabuchi@yone.ac.jp
Table 1. Body Weight Gain, Blood Glucose, HbAIC and Liver TBARS in Rats Fed Experimental Diets for 9 Weeks

<table>
<thead>
<tr>
<th>Body weight (g)</th>
<th>Blood glucose (mg/dL)</th>
<th>Blood HbAIC (%)</th>
<th>Liver TBARS (nmol MDA/g of liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>Gain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>258.4±2.8*</td>
<td>108.0±3.4*</td>
<td>3.0±0.1*</td>
</tr>
<tr>
<td>Control</td>
<td>240.6±1.5*</td>
<td>105.5±6.7*</td>
<td>3.6±0.1*</td>
</tr>
<tr>
<td>GG</td>
<td>244.0±3.5*</td>
<td>111.0±6.5*</td>
<td>3.1±0.1*</td>
</tr>
</tbody>
</table>

Each data value represent the mean ± SEM.

*Values in the same column with different superscript letters significantly differ at p<0.05.

A (8-week-old) [Graph A]
B (18-week-old) [Graph B]

Fig. 1. Increase in Levels of Blood Glucose in Rats as Revealed by Oral Glucose Tolerance Tests before (8-Week-old) and after 9-Week Experimental Diet (18-Week-old).

A: 8-week-old; B: 18-week-old. a,b,c Values in the same column with different superscript letters significantly differ at p<0.05 by Tukey's test. *Values with asterisks are significantly different from the value before glucose loading at p<0.05 by Student's t-test.

Tokyo, Japan). Then, the rats were killed by ether inhalation, and the livers were immediately removed, washed with cold 0.9% saline, and stored at −80°C. The value of thiobarbituric acid-reactive substance (TBARS) in the liver was measured by the method of Ohkawa et al., using a standard of 1,1,3,3-tetraethoxypropane.

The experimental data are presented as the means ± SEM. The differences between groups were considered significant at p<0.05 by Student's t-test and Tukey's test.

As shown in Table 1, body weight gains during the 9-week experimental period were not different among the three groups. The levels of blood glucose and HbAIC in the control group were significantly higher than those in the normal group (p<0.05). In the GG group, the HbAIC level was significantly lower than in the control group (p<0.05), although the blood glucose level was not significantly lowered. HbAIC was glycosylated hemoglobin and was most often used to monitor long-term blood glucose balance, because it reflected the exposure of the hemoglobin molecule to glucose over the life of a red blood cell. These results suggested that the blood glucose of n-STZ rats fed GG cells might be controlled.

Figure 1 presents the results of glucose tolerance tests before and after administration of the experimental diets. The increase in levels of blood glucose before administration of the experimental diets (8-week-old) was almost the same between the control and GG groups (Fig. 1A). However, 9 weeks after the start of the experimental diets (18-week-old), the increase in levels of blood glucose were significantly lower in the GG group than in the control group (p<0.05, Fig. 1B). These results indicated that GG cells could improve glucose tolerance in n-STZ rats. The n-STZ rats showed a gradual exhaustion in beta-cell function induced by postprandial hyperglycemia, as a consequence; insulin secretion was decreased and glucose tolerance was impaired.

As shown in Fig. 2, the serum insulin level 30 min after glucose loading was significantly higher in the GG group than in the control group (p<0.05). Thus, we made the following hypothesis: the administra-
tion of GG cells might suppress the increase in blood glucose during the experiment and might prevent decrease in insulin secretion.

There have been many studies on the antidiabetic effects of dietary fiber\(^1\) and polyphenol,\(^2\) which could suppress glucose absorption from small intestine and prevent rises in blood glucose. We previously observed that lyophilized GG cells could survive in the intestine of rats and change the intestinal flora balance, and L. GG could use the glucose as a source of nutrition in vitro (data not shown). Furthermore, Goldin et al.\(^3\) reported that the viability of L. GG was not affected by the administration in lyophilized form or as fermented milk in human intestine. These findings lead to the hypothesis that GG cells might suppress the glucose absorption and prevent rises in blood glucose, by the use of glucose or change in intestinal environment. In addition, it is possible that the cell wall of GG might also affect glucose absorption like an indigestible fiber. However, because the influence of GG cells on glucose absorption remains controversial, we want to measure the glucose absorption of rats fed GG cells in future experiments.

On the other hand, under hyperglycemic conditions, oxidative stress was produced and caused damage to various tissues, which may play a role in the development of complications in diabetes.\(^1\) As shown in Table 1, the liver TBARS level in the control group was significantly higher than in the normal group \((p<0.05)\). In the GG group, the liver TBARS level was significantly lowered more than the control group \((p<0.05)\), suggesting that the production of oxidative stress might be suppressed, when GG cells improved glucose tolerance. In addition, cell extracts of L. GG have been reported to have antioxidative activity in vitro,\(^4\) which supports the hypothesis that lowering-liver TBARS in the GG group might be due to the antioxidative activity of GG cells. However, the antioxidative effect of GG cells needs further investigation.

In conclusion, this study demonstrated that GG cells lowered the blood HbA\(_1c\), level and improved glucose tolerance in n-STZ rats; and this antidiabetic effect of GG cells might be due to prevention of a decrease in insulin secretion. However, the mechanism by which GG cells prevent decreases in insulin secretion remains unclear. In addition, it is possible that other strains of lactic acid bacteria may show an antidiabetic effect. Further work is needed.

References


