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Effects of Hachimi-jio-gan (Ba-Wei-Di-Huang-Wan) on Intestinal Function in Streptozotocin-induced Diabetic Rats

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We examined the effects of Hachimi-jio-gan (HJ) on the small intestinal function in streptozotocin (STZ)-induced diabetic rats. The rats had free access to pellets containing 1% HJ extract powder for 4 weeks after STZ administration. The intestinal disaccharidase (sucrase and maltase) activity was elevated in STZ-treated rats compared with control rats, whereas it was significantly reduced by HJ administration. This suggested that HJ suppresses or delays monosaccharide production in the small intestinal epithelium. In addition, the intestinal mucosal weights and DNA contents that were significantly increased in the STZ-treated rats were restrained to the control level by HJ treatment. Simultaneously, we examined the changes in the plasma levels of glucagon-like peptide 2 (GLP-2), which is a trophic factor specific for the intestine. The plasma GLP-2 levels significantly increased in the STZ-treated rats, whereas HJ decreased the plasma GLP-2 levels. Thus intestinal mucosal weights and DNA contents correlated with plasma GLP-2 levels in diabetes-associated bowel growth. These results suggest that HJ may normalize or suppress the small intestinal disaccharidase activity and the epithelial cell proliferation mediated by GLP-2 in the animal model rats.

Key words——Hachimi-jio-gan (Ba-Wei-Di-Huang-Wan); small intestine; disaccharidase activity; glucagon-like peptide 2 (GLP-2); streptozotocin; rat

INTRODUCTION

The life prognosis of diabetic patients has been improved by the recent advancements in diabetes treatment. However, preventive measures against chronic complications such as diabetic neuropathy, retinopathy, nephropathy, and in addition, arteriosclerosis that leads to blood vessel dysfunction, have become major issues associated with long-duration diabetes.[1,2] Furthermore, diabetic patients frequently develop digestive symptoms that are associated with abnormal motility and secretion in the entire gastrointestinal tract, and the abnormal function of the digestive tube is demonstrated in patients who do not present with the subjective symptoms of diabetes mellitus.[3] A variety of factors such as diabetic autonomic neuropathy, microcirculation dysfunction, hyperglycemia, electrolyte imbalance, increased susceptibility to infection, and abnormal production and secretion of gastrointestinal peptides and neurotransmitters are responsible for these abnormal symptoms.[1,4,5] Moreover, intestinal mucosal hypertrophy[6,7] and the related increase in disaccharidase activity is observed in both animals and humans.[8,9]

It is reported that one of the traditional Kampo prescriptions, namely, Hachimi-jio-gan (HJ), has an antidiabetic action that ameliorates hyperglycemia and protects against diabetic nephropathy in experimental diabetic model rats.[10,11] In this study, we examined the effects of HJ on intestinal function using streptozotocin (STZ)-induced diabetic model rats. The sucrase and maltase activities in the intestinal mucosa were measured as indices of digestive function measurement, and the mucosal weights, DNA contents and plasma Glucagon-like peptide 2 (GLP-2) levels were measured as indices of intestinal mucosa proliferation. GLP-2 increases crypt cell proliferation and decreases the rate of apoptosis in small intestinal epithelium.[12] We demonstrated that

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correlations were found between plasma GLP-2 levels and mucosal weight and DNA in rats with intestinal injury.[13] And also, GLP-2 administration increased body mass and improved nutrient absorption in a pilot study of human subjects with short bowel syndrome.[14]

MATERIALS AND METHODS

Chemicals HJ (TJ-7; Tsumura Inc., Tokyo, Japan) is composed of 8 herbal extracts: Rehmanniae Radix (6.0), Corni Fructus (3.0), Dioscoreae Rhizoma (3.0), Alismatis Rhizoma (3.0), Hoelen (3.0), Moutan Cortex (2.5), Cinnamomi Cortex (1.0), and Aconiti Tuber (0.5).

Animals Male Wistar rats (Clea Japan, Inc., Tokyo, Japan) weighing 180–190 g were used. Rats were maintained for 1 week on the MF diet® (Oriental Yeast, Tokyo, Japan). The animals had *ad libitum* access to rat chow and water and were kept in a room maintained at 23±2℃ with a 12 h/12 h light/dark cycle. All the experimental procedures were conducted in accordance with the Osaka University Medical School Guidelines for the Care and Use of Laboratory Animals.

Animal Experiment The rats were randomly divided into 3 groups of 6 or 7 animals each. After an overnight fast, 200 μl of STZ solution dissolved in 5 mM citrate buffer (pH 4.5) was injected into the tail vein at a dose of 40 mg/kg. In the control group, the same volume of the buffer was injected instead. Immediately after the injection, the control group and the STZ group were given the pellet chow without HJ, while the STZ+HJ group was given (*ad libitum*) the pellet chow that included 1% HJ extract powder. The HJ powder was mixed with the chow (weight ratio, HJ : chow=1 : 99) and molded into the shape of a pellet. At the end of the 4-week period, blood was taken directly from the vena cava for the GLP-2 assay under ether anesthesia. The blood samples were drawn into vials on ice that contained ethylenediaminetetraacetic acid (EDTA) (2 mg) and aprotinin (400 KIU) per ml of blood. Immediately after blood centrifugation, the plasma samples were stored at −30℃ until assay. During laparotomy, sections of the jejunum and the middle segment of the ileum were quickly excised and flushed with iced saline solution. Excess liquid was removed by blotting the excised tissue segments with filter paper, and the segments were then immediately frozen at −80℃ until assay.

Mucosal Treatment The jejunal segments and the middle segments of the ileum were thawed and opened longitudinally, gently patted dry, and weighed. The mucosa was scraped off with a glass slide and weighed. The mucosal samples from each segment were homogenized on ice with a 5-fold weight of ice-cold saline using a homogenizer (Model BM-1, Nihon Seiki Seisakusho, Tokyo, Japan).

Intestinal Mucosal Disaccharidase Activity Sucrase and maltase activities in the rat ileum were determined by a modification of the method of Dahlquist.[15] Briefly, the homogenates were centrifuged at 10000 g at 4℃ for 30 min. The homogenate supernatants were diluted and added to an equal volume of 0.1 M sodium maleate buffer (pH 6.0) containing 56 mM sucrose or maltose and incubated for 1 h at 37℃. The mixtures were then added to the glucose oxidase-peroxidase reagents that contain *o*-dianisidine as a chromogen, and the absorbance was measured at 420 nm. The protein concentration of each homogenate sample was determined by the method of Lowry et al. using bovine serum albumin as a standard.[16] The disaccharidase activity was expressed as U/min/mg protein.

Intestinal Mucosal DNA Content The homogenate in the jejunal segments was used to assess the DNA contents after dilution with saline. Mucosal DNA contents were measured by the method of Le Pecq and Paoletti using polymerized calf thymus DNA (Sigma Chemical Co., MO, USA) as a standard.[17]

Assay of Rat GLP-2 The enzymeimmunoassay (EIA) system for the measurement of rat plasma GLP-2 levels was developed using synthetic rat GLP-2 (1–33) as a standard antigen (0.137–100 ng/ml), biotinylated rat GLP-2 as a labeled antigen (0.42 ng/ml), and anti-rat GLP-2 serum RY652 (1 : 150000 dilution) as an antibody (YK140 Rat GLP-2 EIA, Yanaihara Inc., Shizuoka, Japan).[18] In brief, the assay was performed in 96-well microtiter plates (Maxisorp, Nunc, Denmark) adsorbed with goat anti-rabbit IgG (1 : 200 dilution, ICN Pharmaceuticals, CA, USA). After these agents and samples were added to the wells and incubated overnight at 4℃, a streptavidin-horseradish peroxidase solution (1 : 10000 dilution, Carbiochem, CA, USA) and an *o*-phenylenediamine solution (OPD, ICN Pharmaceuticals, CA, USA) were added. The absorbance of each well at 492 nm was measured using a microplate read-

er (Labsystems Multiskan MS, Labsystems Co., Ltd., USA).

Statistical Analysis All data are expressed as means±SD. Tukey-Kramer test was used for the statistical analysis. The values were considered to be significantly different when the p value was less than 0.05.

RESULTS

Intestinal Disaccharidase Activity Figure 1 shows the effects of HJ on the sucrase and maltase activities in the rat ileal mucosa. The sucrase and maltase activities were 0.53±0.12 and 5.30±0.75 U/min/mg protein, respectively, in the control group and 1.61±0.44 and 9.30±1.84 U/min/mg protein, respectively, in the STZ group. Both activities increased significantly ($p<0.01$) in the STZ group when compared with the control group. On the other hand, the sucrase and maltase activities were 0.89±0.29 and 6.85±1.54 U/min/mg protein, respectively, in the STZ+HJ group indicating that the enzyme activities that had been elevated by the STZ administration were significantly suppressed with HJ addition.

Intestinal Mucosal Weights and DNA Contents Figure 2 shows the effects of HJ on jejunal mucosal weights and DNA contents in the STZ-induced diabetic rats. The mucosal weight of the STZ group was 24.7±2.0 mg/cm; this was significantly greater than that of the control group, i.e., 13.5±2.4 mg/cm ($p<0.01$), thus confirming mucosal hypertrophy in the STZ-induced diabetic rats (Fig. 2(A)). The mucosal weight of the STZ+HJ group was 11.9±2.1 mg/cm,

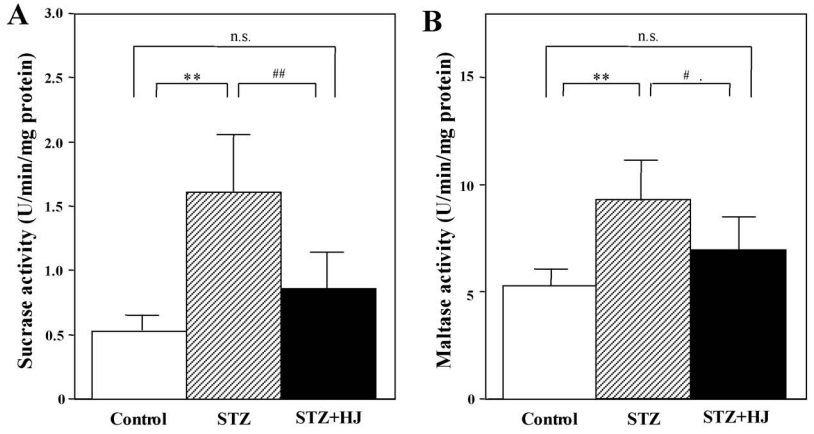

Fig. 1. Effects of HJ on Sucrase and Maltase Activity in STZ-induced Diabetic Rats

A: sucrase activity in rat ileum and B: maltase activity in rat ileum. Each values represent the mean±S.E. $^{**}p<0.01$ compared with control rats, $^{\sharp}p<0.05$, $^{\sharp\sharp}p<0.01$ compared with STZ rats.

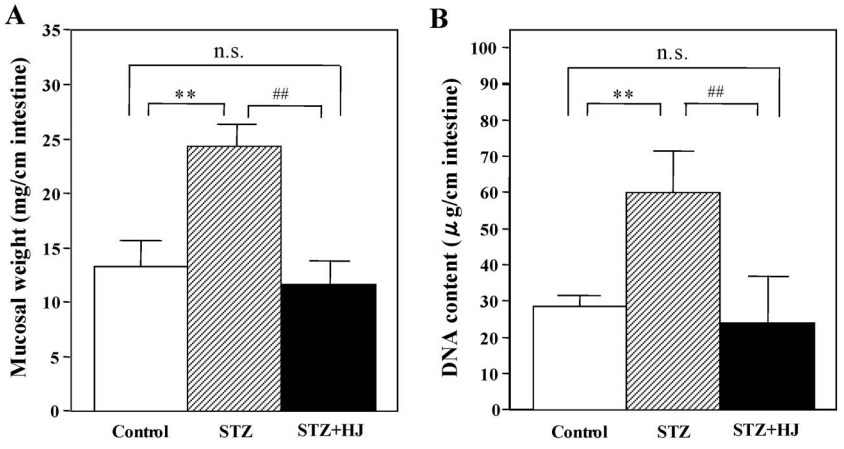

Fig. 2. Effects of HJ on Mucosal Weight and DNA Content in STZ-induced Diabetic Rats

A: mucosal weight in rat jejunum and B: DNA content in rat jejunum. Each value represents the mean±S.E. $^{**}p<0.01$ compared with control rats and $^{\sharp\sharp}p<0.01$ compared with STZ rats.

and HJ significantly suppressed the mucosal weight ($p<0.01$). The DNA content of the jejunal mucosa of the STZ group was $60.7\pm11.4\,\mu g/cm$; this was significantly higher than that of the control group, i.e., $28.9\pm2.80\,\mu g/cm$ ($p<0.01$) (Fig. 2(B)). These results agreed with the increase in the mucosal weight. Further, in the STZ+HJ group, the DNA content of the jejunal mucosa was $24.9\pm12.5\,\mu g/cm$; this value was close to that of the control group. HJ strongly restrained the increase in the DNA content caused by the STZ administration.

Plasma GLP-2 Levels Figure 3 shows the effects of HJ on the plasma GLP-2 levels in STZ-induced diabetic rats. The plasma GLP-2 level of the control group was 3.57 ± 0.37 ng/ml while that of the STZ group was significantly higher at 4.41 ± 0.76 ng/ml ($p<0.05$). These results were correlated with the significant increase in the mucosal weights and DNA contents of the STZ group. However, the plasma GLP-2 level in the STZ+HJ group was significantly decreased (3.43 ± 0.72 ng/ml) and was close to the control level ($p<0.01$).

DISCUSSION

In the present study, we investigated the effects of HJ on the disaccharidase activity and epithelial cell proliferation of the small intestinal mucosa in the diabetic model rat induced by STZ, which destroys β-cells specifically. Disaccharidase activity has been shown to be increased in diabetic patients.[8] Further, in various diabetic model animals, hyperplasia of the intestinal mucosa has been reported.[6,7] As a result, the production of glucose and its absorption in the small intestine increases remarkably in the diabetic condition and may amplify the blood glucose level.

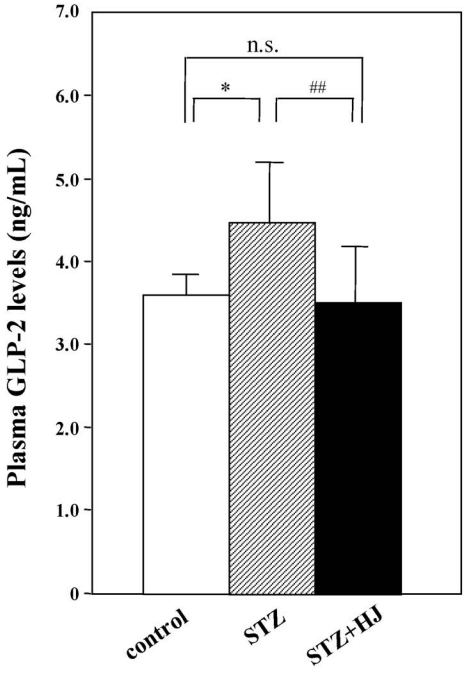

Fig. 3. Effects of HJ on Plasma GLP-2 Levels in STZ-induced Diabetic Rats

Each values represent the mean±S.E. $^{*}p<0.05$ compared with control rats and $^{\#\#}p<0.01$ compared with STZ rats.

Our findings are in agreement with the report that a significant increase in sucrase and maltase activities is linked to the increase in the intestinal mucosal weights and DNA contents of STZ-induced diabetic rats.[6] And also, we demonstrated that HJ reduced the activity of sucrase and maltase in the small intestinal mucosal epithelium of diabetic rats. On the other hand, HJ reduced blood glucose levels by increasing plasma and pancreatic insulin levels and depressing GLUT 2 protein expression during the same period, i.e., 4 weeks after HJ administration to the STZ-treated rats.[19] There is a possibility that these findings contribute partly to the hypoglycemic action of HJ.

As discussed above, intestinal mucosal hyperplasia was observed in the diabetic condition of both experimental animals and humans.[6,8] In the model rats, the jejunal mucosal weights of the STZ group were increased by approximately 2-fold when compared with that of the control group. Further, the increment in the mucosal weights of the intestinal segments was coupled to the increase in the DNA contents of the same segments. Consequently, the enlargement of the intestinal mucosa in diabetic rats may also be an appropriate compensation response to the hyperphagia that accompanies diabetes mellitus with the elevated activity of disaccharidases.

Next, we examined the effects of HJ on the plasma GLP-2 levels by intestinal growth in STZ-induced diabetic rats. GLP-2 has intestinotrophic effects and belongs to the proglucagon-derived peptide family.[20,21] GLP-2 are secreted in a nutrient-dependent fashion by the enteroendocrine L cells of the intestines.[22] Fisher et al. reported that the plasma GLP-2 levels rose in the STZ-treated rats.[7] The plasma GLP-2 levels also reflect the GLP-2 concentrations in the intestinal mucosal tissues.[20] The present investigation demonstrated that the plasma GLP-2 levels were connected with the increase in the mucosal weights and

DNA contents of the STZ group. Furthermore, these increased mucosal weights and DNA contents were significantly decreased by HJ administration. At the same time, HJ significantly lowered plasma GLP-2 levels, implying that HJ suppresses mucosal hyperplasia by restraining the GLP-2-mediated proliferation.

In this research, we clarified that HJ improved the small intestinal disaccharidase activity and epithelial cell proliferation in the diabetic model rats. Further research is clearly needed in order to determine how HJ controls intestinal function and regulates food intake.

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