Original Article

Effects of the herbal medicine Hachimi-jio-gan (Ba-Wei-Di-Huang-Wan) on insulin secretion and glucose tolerance in type 2 diabetic Goto-Kakizaki rats

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ABSTRACT: Hachimi-jio-gan (HJ) is a Chinese medicine that has been widely used for the treatment of nephrotic syndromes, hypertension, and diabetes mellitus. We reported that HJ lowers plasma glucose in type 1 diabetic rats. We investigated the effects of HJ on diabetic hyperglycemia and insulin secretion in type 2 diabetic Goto-Kakizaki (GK) rats. Eight-week-old diabetic GK rats were given free access to pellets containing 1% HJ extract powder for 14 weeks. HJ consumption increased the food intake and body weight of these rats in comparison to control rats. HJ may control the body weight loss observed in GK rats. HJ also reduced hyperglycemia in diabetic GK rats, and it significantly increased insulin secretion in non-fasting GK rats over the experimental period. In oral glucose tolerance tests, HJ significantly improved the insulin response at 30 min and reduced the plasma glucose level at 60 min after glucose administration ($p < 0.05$). Ten weeks after administration, the plasma leptin levels significantly increased in the HJ group rats. These results demonstrate that in diabetic GK rats, HJ decreased the level of postprandial glucose *via* enhanced insulin secretion coupled with the regulation of food intake by leptin.

Keywords: Hachimi-jio-gan (HJ), hyperglycemia, insulin, body weigh, Goto-Kakizaki rat

1. Introduction

The herbal medicine Hachimi-jio-gan (HJ) (Ba-Wei-Di-Huang-Wan) has been widely used for the treatment of hypertension, nephrotic syndromes, glomerulonephritis, and diabetes mellitus since the late middle ages. In Japan, the medicinal uses of HJ are provided in the package insert of the HJ extract. Animal studies have shown that Rehmanniae radix, Corni fructus, and Hoelen lower blood sugar (*1*). However, HJ does not contain ginseng, which is frequently used to treat diabetes in the clinical setting. Various human and animal studies have been conducted on the therapeutic effects of HJ (*2-5*). Most of these studies have investigated the effects of HJ on renal damage, including diabetic nephropathy, and the mechanism underlying these effects (*6-8*). We have reported the antidiabetic effects of HJ in rats in which various symptoms, including persistent hyperglycemia, were induced by streptozotocin (STZ) administration (*9-11*). HJ is used in the treatment of diabetes-associated symptoms such as dry mouth, thirst, and polyuria (*12*). However, despite the fact that clinical and experimental studies have provided evidence for the various beneficial effects of HJ, to date, only a few scientific studies have presented corroborating evidence on such subjective symptoms.

In the present study, we used spontaneouly diabetic Goto-Kakizaki (GK) rats as animal models of genetic predisposition to non-insulin-dependent diabetes mellitus (NIDDM) (*13*). In GK rats, glucose-stimulated insulin secretion is reduced, and glucose tolerance is impaired. Moreover, GK rats with impaired insulin secretion do not become obese and do not develop hyperlipidemia (*14*). These characteristics of GK rats are similar to those observed in NIDDM patients (*15-17*). The GK rat is thus considered a suitable model for the investigation of the effects of HJ on insulin secretion and glucose tolerance. In this study, we investigated the effects of HJ on diabetes symptoms, including hyperglycemia, in diabetic GK rats. We also examined the effect of HJ administration on the food intake and body weight of GK rats.

2. Materials and Methods

2.1. *Animals*

Male GK and Wistar rats (Japan SLC Inc., Shizuoka, Japan) weighing 180-190 g were used in this study. The rats were maintained for 1 week on a standard pellet

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diet (MF diet; Oriental Yeast, Tokyo, Japan). They were kept in a room maintained at 22 ± 2°C with a 12 h/12 h light/dark cycle (diurnal time, 8:00-20:00) and had free access to rat chow and water. All experimental procedures were conducted in accordance with the Osaka Ohtani University Guidelines for the Care and Use of Laboratory Animals.

2.2. *Drugs*

The HJ extract was obtained from Tsumura & Co. Ltd. (Tokyo, Japan). The composition of HJ is as follows: Rehmanniae Radix (*Rehmannia glutinosa* Libosch), 6 g; Corni Fructus (*Cornus officinalis* Sieb et Zucc.), 3 g; Dioscoreae Rhizome (*Dioscorea japonica* Thunb.), 3 g; Alismatis Rhizome (*Alisma orientale* Juzep.), 3 g; Hoelen (*Poria cocos* Wolf), 3 g; Moutan Cortex (*Paeonia suffruticosa* Andrews), 2.5 g; Cinnamomi Cortex (*Cinnamomum cassia* Blume), 1 g; and Aconiti Tuber (*Aconitum carmichaeli* Debx), 0.5 g. Aqueous extracts were prepared from the crude drugs and spray dried to obtain the extract powder. The three-dimensional HPLC profile of HJ provided by Tsumura Inc. is shown in Figure 1.

2.3. *Treatment of animals and preparation of sample*

The GK rats were randomly divided into 2 groups of 5 animals each. The control group was given pellet chow without HJ, while the HJ group was given pellet chow containing 1% HJ extract powder. The pellets and tap water were provided *ad libitum*. The food intake and body weight of the rats were measured every week. Every week for the next 14 weeks, non-fasting blood samples were collected from the jugular vein at 10:00 A.M. and stored in chilled tubes containing EDTA-2Na (at a final concentration of 0.03 mM) and dipeptidyl peptide IV (DPP-IV) inhibitor (10 μL/mL blood) (Millipore, MO, USA). At the end of the 14-week period, blood samples were collected from the inferior vena cava under ether anesthesia, and the plasma was immediately separated by centrifugation.

2.4. *Assay of plasma glucose levels*

Plasma glucose levels were determined by using a commercial reagent (Glucose CII-Test Wako; Wako Pure Chemical Industries Ltd., Osaka, Japan).

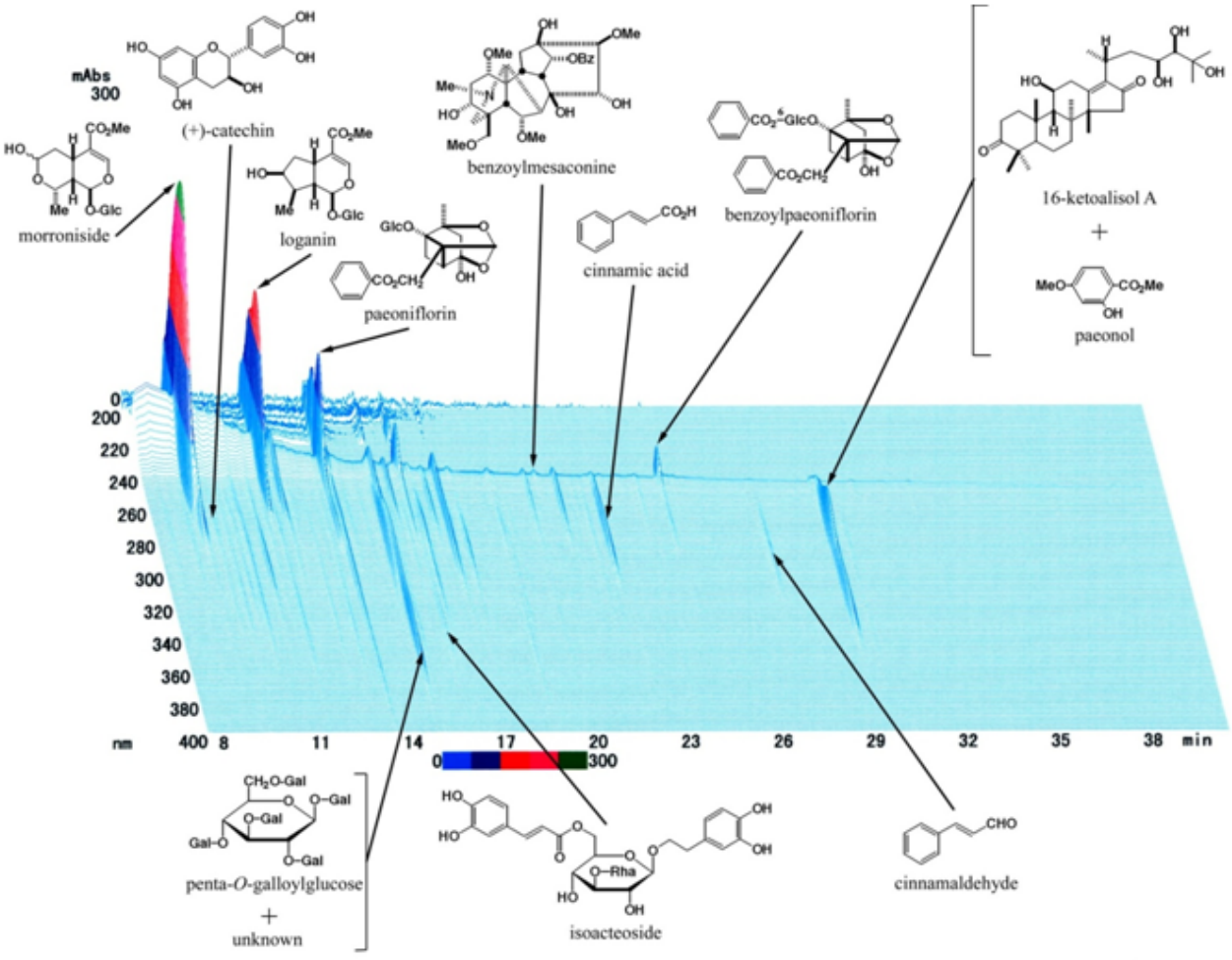

Figure 1. Three-dimensional HPLC profile of HJ.

2.5. *Immunoassay of plasma hormone levels*

Plasma immunoreactive insulin levels were measured by using a commercial radioimmunoassay kit (Insulin Eiken RIA kit; Eiken Chemical Co. Ltd., Tokyo, Japan). In each assay tube, 25 µL of standard insulin or the unknown sample, 50 µL of guinea pig anti-insulin plasma, and 50 µL of ^{125}I-labeled insulin were mixed, and the mixture was incubated at 2-8°C for 24 h. To the mixture, 50 µL of goat anti-guinea pig γ-globulin plasma was added; this was followed by further incubation at 2-8°C for 30 min and centrifugation at 3,000 rpm at 4°C for 30 min. The supernatant was aspirated, and the radioactivity of the precipitate was counted in a gamma counter. Plasma leptin levels were measured by using the Rat Leptin-HS ELISA Kit (YK051; Yanaihara Institute Inc., Shizuoka, Japan).

2.6. *Oral glucose tolerance test*

In the final week of treatment, an oral glucose tolerance test (OGTT) was performed on the GK (age, 23 weeks) rats after 16 h of starvation. The control blood sample was collected from the jugular vein at 0 min and stored in chilled tubes containing EDTA-2Na. Glucose (2 g glucose/kg body weight) was administered orally *via* a stomach tube, and blood was collected from the jugular vein at 0, 30, 60, and 120 min. The plasma glucose and insulin levels were determined for each time point by using the same reagents.

2.7. *Data analysis*

Except for the amount of food intake, all experimental data are expressed as mean ± standard deviation (S.D.). The differences between the mean values were statistically analyzed by using Tukey-Kramer test and Student's unpaired *t*-test with a significance level of $p < 0.05$.

3. Results

3.1. *Food intake and body weight*

The changes in the food intake and body weights are showed in Figure 2. The body weights of rats from the HJ group significantly increased after the fifth week compared with the control group ($p < 0.05$). The mean daily food consumption and body weights of the HJ-administered rats were higher than those of the control rats, but were not beyond those of the normal rats.

3.2. *Non-fasting plasma glucose levels*

Figure 3 shows the changes in non-fasting plasma glucose levels during the period of HJ administration. The plasma glucose level for the HJ group rats had a tendency to lower than that for the control group rats except weeks 1 and 12. This tendency was particularly marked in weeks 5, 7, 8, 9, and 13 ($p < 0.05$).

3.3. *Plasma insulin levels*

Figure 4 shows the changes of plasma insulin levels observed during the study period. In the HJ group rats, the non-fasting plasma insulin level significantly

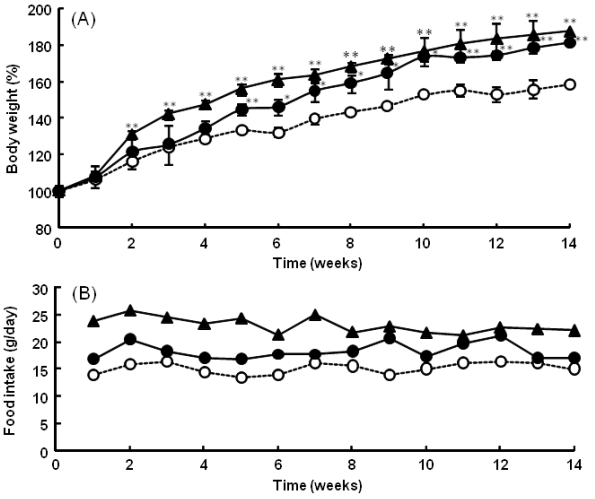

Figure 2. Effects of 14-week treatment with HJ on body weight (A) and mean food intake amount per cage (g/day) (B) in GK rats. ▲, normal rats treated vehicle (Normal group); ○, GK rats treated with vehicle (Control group); ●, GK rats treated with HJ (HJ group). * $p < 0.05$, ** $p < 0.01$ compared with the control group. Data are mean ± S.D. ($n = 5$).

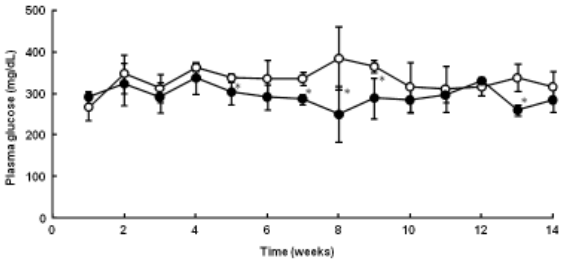

Figure 3. Effects of HJ on non-fasting plasma glucose levels during 14 weeks of treatment in GK rats with or without HJ. ○, GK rats treated with vehicle (Control group); ●, GK rats treated with HJ (HJ group). * $p < 0.05$ compared with the control group. Data are mean ± S.D. ($n = 5$).

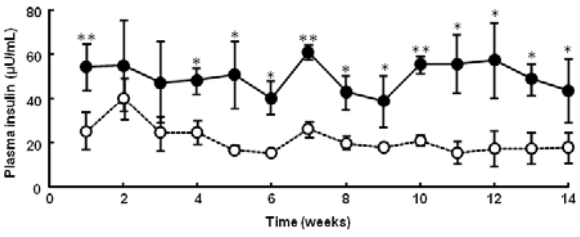

Figure 4. Effect of HJ on non-fasting plasma insulin levels during 14 weeks of treatment in GK rats with or without HJ. ○, GK rats treated with vehicle (Control group); ●, GK rats treated with HJ (HJ group). * $p < 0.05$, ** $p < 0.01$ compared with the control rats. Data are mean ± S.D. ($n = 5$).

increased throughout the experiment except for the second and third weeks. The plasma insulin level of the HJ group rats was approximately twice as high as that of the control group rats.

3.4. *Oral glucose tolerance test*

Figure 5 shows the changes in OGTTs performed 14 weeks after treatment. The control group exhibited the typical pattern observed in diabetes: glucose administration caused a quick increase in the plasma glucose, and it remained high. However, at 60 min, the plasma glucose was found to be significantly lower in the HJ group rats than in the control group rats (459 ± 19 *vs*. 413 ± 10 mg/dL, $p < 0.05$). In contrast, the plasma insulin levels of the HJ group rats increased markedly at 30 min (7.4 ± 0.2 *vs*. 14.5 ± 1.1 μU/mL, $p < 0.05$) and returned to the control levels after 120 min (Figure 5B).

3.5. *Plasma leptin levels*

Figure 6 shows the changes in the non-fasting plasma leptin levels during the study period. In the control group rats, these levels did not change significantly during the study period. In the HJ group rats, however, they increased gradually along with HJ administration, and in the 10th, 12th, and 14th weeks, they were significantly higher than those of the control group rats (10th and 14th week, $p < 0.05$; 12th week, $p < 0.01$).

4. Discussion

Diabetic GK rats have been reported to have i) decreased insulin secretory response of the pancreatic ß cells to glucose and ii) insulin resistance – the 2 main characteristics of NIDDM (*18*). In contrast to many other rodent models of NIDDM, GK rats do not become obese and do not develop hyperlipidemia. These characteristics are similar to the typical Asian-type diabetes (*17*,*19*). The GK rat is thus considered to be a suitable model for the investigation of the effect of HJ on insulin secretion and glucose tolerance.

HJ is a Chinese medicine that has been widely used in the treatment of diabetes for a long period of time. Although few clinical studies have developed the herbal formula to lower blood glucose levels, HJ is administered to diabetic patients. It has been

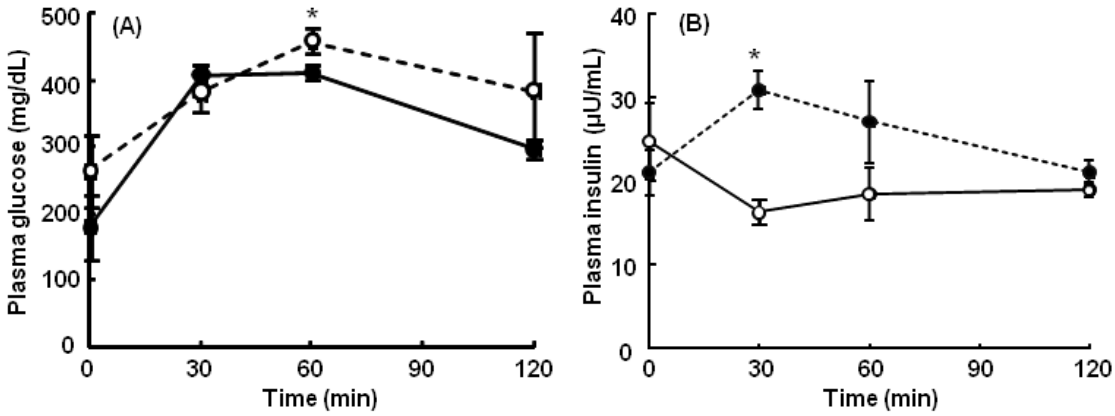

Figure 5. Effects of HJ on plasma glucose (A) and insulin (B) levels in oral glucose tolerance test after 14 weeks of treatment in GK rats with or without HJ. ○, GK rats treated with vehicle (Control group); ●, GK rats treated with HJ (HJ group). * $p < 0.05$ compared with the control rats. Data are mean ± S.D. ($n = 5$).

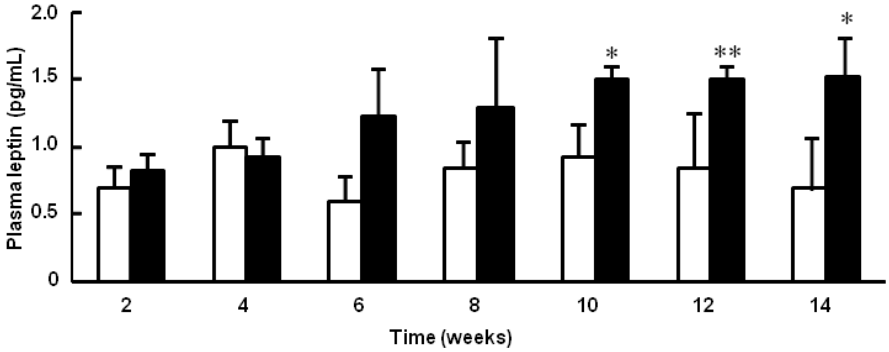

Figure 6. Effects of HJ on plasma leptin levels during 14 weeks of treatment in GK rats with or without HJ. □, GK rats treated with vehicle (Control group); ■, GK rats treated with HJ (HJ group). * $p < 0.05$, ** $p < 0.01$ compared with the control rats. Data are mean ± S.D. ($n = 5$).

clinically established that while HJ does not improve hyperglycemia, it can abate diabetes-related subjective symptoms such as dry mouth, frequent thirst, frequent urination, and fatigue. Moreover, in recent years, many studies have been conducted on the prevention and treatment of diabetic complications (*20*,*21*), and HJ has been found to be effective in some patients who do not respond to modern medicine. However, these studies are not sufficient to support these therapeutic effects of HJ.

We have previously reported the antidiabetic effects of HJ in STZ-induced diabetic rats as a model of insulin-dependent diabetes mellitus (IDDM) (*9*). HJ significantly increased the plasma and pancreatic insulin levels in these rats. Although there was no increase in the number of pancreatic β cells in the islets of Langerhans, HJ induced an increase in insulin production and secretion by the residual pancreas and also significantly reduced the synthesis of glucose transporter-2 protein, which is involved in glucose uptake and its release, in the liver. Hence, these results suggest that HJ affects not only the production and secretion of insulin but also the release of glucose from the liver. Further, it alleviates subjective symptoms and prevents complications of diabetes mellitus (*8*,*10*,*22*). In these our reports, the administration of 1% HJ contents to the animals substantially exhibited the anti-diabetic effects.

We used GK rats as a model of human NIDDM to ascertain the effects of HJ on hyperglycemia. In order to examine the effect of HJ in glycemic control, we studied its effects on insulin secretion in diabetic GK rats. HJ administration in GK rats lowered the non-fasting plasma glucose levels throughout the study period and significantly suppressed it in weeks 5, 7, 8, and 13 (Figure 3). The increasing of leptin levels after weeks 10 may directly influence an antihyperglycemic action by insulin. However, HJ also significantly increased plasma insulin levels over the experimental period in these rats (Figure 4).

The OGTTs revealed that the tolerance to glucose had improved in the HJ group rats compared to the control group rats. The plasma glucose levels at 60 min also decreased significantly ($p < 0.05$) in the HJ group rats (Figure 5A). The plasma insulin levels at 30 min were significantly higher in the HJ group rats than in the control group rats (Figure 5B). In addition, the HJ group showed a rapid increase at 30 min in the plasma insulin levels that returned to the control levels after 120 min and HJ restored the impaired insulin secretion in GK rats. HJ improved glucose tolerance at 14 weeks after HJ administration in GK rats. And also, the HOMA-R score (index of insulin resistance) in the HJ group rats at 14 weeks was significantly lower than that in the control rats (shown not data), indicating that insulin resistance was ameliorated in HJ-treated rats.

In the present study, the body weights and food intake increased in the HJ group (Figure 2). We found that the body weights of the HJ group rats increased significantly after the fifth week ($p < 0.05$), and these weights were similar to those of normal rats (Wistar rats). HJ normalized the body weight of GK rats. Thus, HJ administration resulted in an increase in the body weight and food consumption, in addition to an improvement in insulin secretion. We thus investigated the effects of HJ on plasma leptin levels, since leptin is involved in food intake. The plasma leptin levels increased in the HJ group rats compared to the control group rats. We think that the HJ group rats did not become obese due to this involvement of leptin. However, further studies are required to clarify this issue. The body weights of the genetically diabetic GK rats were reduced as compared to that of normal (Wistar) rats of the same age. But the HJ group rats exhibited a moderate increase in body weight when the plasma insulin and leptin levels increased. Plasma leptin levels were lower in GK rats than in Wistar rats and plasma leptin levels in the HJ group rats were nearly the same as those in Wistar rats reported by Huang *et al.* (*23*). However, it remains to be elucidated whether leptin sensitivity is altered by HJ administration.

In conclusion, HJ is a useful antidiabetic agent which shows improvement of glucose tolerance by glucose stimulated insulin secretion, and body weight loss in spontaneously diabetic GK rats.

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