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GOSHAJINKIGAN (CHINESE HERBAL MEDICINE NIU-CHE-SEN-QI-WAN) IMPROVES INSULIN RESISTANCE IN DIABETIC RATS VIA THE NITRIC OXIDE PATHWAY

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ABSTRACT

Goshajinkigan (GJG), an aqueous extract of a combination of 10 herbal medicines, is widely used for the treatment of diabetic neuropathy in Japan. In this study, the effect of GJG on insulin-induced glucose disposal in normal and streptozotocin (STZ) diabetic rats was analyzed using the euglycemic clamp technique. Male Wistar rats, aged 9 weeks, were randomly assigned to six groups: group NS, normal rats receiving saline; group NG, normal rats receiving GJG (800 $mg \cdot kg^{-1} \cdot day^{-1}$, p.o.); group NGL, normal rats receiving GJG + N^G-monomethyl-L-arginine (L-NMMA, 1 $mg \cdot kg^{-1} \cdot min^{-1}$, i.v.); group DS, diabetic rats receiving saline; group DG, diabetic rats receiving GJG; group DGL, diabetic rats receiving GJG + L-NMMA. After daily oral administrations of saline or GJG for one week, euglycemic clamp experiments were performed. The metabolic clearance rates of glucose (MCR) in the DS, DG, and DGL groups (8.7 ± 2.9, 18.2 ± 2.5, and 8.1 ± 1.8 $ml \cdot kg^{-1} \cdot min^{-1}$, respectively) were significantly lower than those in the NS, NG, and NGL groups (24.1 ± 4.5, 24.5 ± 3.1, and 22.2 ± 2.1 $ml \cdot kg^{-1} \cdot min^{-1}$, respectively). In addition, the MCR in the DG group was significantly higher than that in the DS and DGL groups, while no significant difference was detected among the NS, NG, and NGL groups. Furthermore, the amelioration of insulin resistance by GJG in diabetic rats was hampered by L-NMMA infusion. These results suggest that daily GJG administrations ameliorate insulin resistance in STZ-diabetic rats, and that the nitric oxide pathway may mediate the effect of GJG.

Key Words: Kampo medicine, Goshajinkigan, Insulin resistance, Diabetes, Nitric oxide

INTRODUCTION

Herbal medicines—well-known as Kampo formulations—have been used for thousands of years to treat diseases and promote general health. Given that plant extracts have only mild and gradual effects with few associated side effects, Kampo formulations have been proven useful for the treatment of chronic illnesses, including diabetes mellitus.

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More than 400 traditional plant therapies for diabetes mellitus have been documented in different regions of the world. However, since only a few of these medicines have undergone scientific and/or medical investigation, the World Health Organization (1980) has recommended that the effectiveness of traditional Kampo treatments for diabetes mellitus should be examined.

Medicines used in traditional Japanese Kampo practice are aqueous extracts of mixtures of unrefined, crude plant materials. Kampo formulations such as Xiaoke,[1] Seishinrenshiin,[2] and To-Kai-San[3] have been reported to possess hypoglycemic effects in animal models of diabetes. In addition, Goshajinkigan (GJG), a mixture of ten raw herbs (Table 1), has been used since the Han period in China to treat numbness, cold sensation in the extremities, and pain in people with diminished physical strength. Furthermore, in recent years, GJG has been extensively used to treat diabetic neuropathy in Japan[4-7], and the mechanism underlying its effects appears to involve the nitric oxide (NO) pathway.[4-11] Moreover, a single administration of GJG has proven effective in improving insulin resistance in diabetic rats.[12] However, to the best of our knowledge, no animal studies have been conducted to date on the potential insulin resistance ameliorating effect of consecutive GJG administrations.

The purpose of this study was to investigate the effect of continued GJG treatment on insulin action in rats and to determine the mechanism that mediates GJG-induced improvements in insulin sensitivity.

MATERIALS AND METHODS

Materials

Spray-dried GJG powder was obtained from Tsumura & Co. (Tokyo, Japan). Streptozotocin (STZ), N^G-monomethyl-L-arginine (L-NMMA), and insulin were purchased from Sigma-Aldrich Co. (St. Louis, USA), Calbiochem (Darmstadt, Germany), and Novo Nordisk (Bagsværd, Denmark), respectively.

Animals and catheterization procedures

Thirty male Wistar rats, age 8 weeks (210–230 g), were purchased from Chubu Kagaku Shizai Co. (Nagoya, Japan) and maintained in an environmentally-controlled room with temperature set at 23°C and light on a 12:12-h light/dark cycle. Animals had free access to water and a standard rodent diet (CLEA Japan Inc., Tokyo, Japan).

Table 1 Composition of Goshajinkigan (Niu-Che-Sen-Qi-Wan)

Component	Amount (%)
Rehmanniae radix	17.9
Achyranthis radix	10.7
Corni fructus	10.7
Dioscoreae rhizoma	10.7
Plantaginis semen	10.7
Alismatis rhizoma	10.7
Hoelen	10.7
Moutan cortex	10.7
Cinnamomi cortex	3.6
Aconiti tuber	3.6

The herbal extract used in this study was an unprepared bulk powder of TJ-107 (Tsumura & Co., Tokyo, Japan).

After one week of acclimation, rats were randomly assigned to six groups: group NS, normal rats receiving saline; group NG, normal rats receiving GJG (800 $mg \cdot kg^{-1} \cdot day^{-1}$, p.o.); group NGL, normal rats receiving GJG + L-NMMA (1 $mg \cdot kg^{-1} \cdot min^{-1}$, i.v.); group DS, diabetic rats receiving saline; group DG, diabetic rats receiving GJG; group DGL, diabetic rats receiving GJG + L-NMMA. The GJG dose was approximately 10-fold higher than that used in clinical practice.[2,4,6]

Overnight (16 hr) fasted rats were anesthetized by an intraperitoneal injection of sodium pentobarbital (50 $mg \cdot kg^{-1}$). Vessels of the neck region were exposed, and the ends of silicon catheters (0.2 mm inner and 0.37 mm outer diameters, Silascon, Dow Corning Co., Midland, USA) were inserted into the right jugular vein and the left carotid artery. The free ends of both catheters were then tunneled under the skin and exteriorized through a small incision in the nape of the neck. Diabetes was induced in rats of groups DS, DG, and DGL by injecting STZ (50 $mg \cdot kg^{-1}$) through the jugular vein catheter. Starting 2 days after surgery, GJG or saline was administered daily at 17:00 h, using an oral feeding needle, for one week (until two days before the euglycemic clamp study). Animal care conformed to the standards set by the "Principles of Laboratory Animal Care" (NIH publication number 85–23, revised 1985) and the "Guidelines for Animal Experimentation" (Nagoya University, 1988).

Euglycemic clamp procedures

The euglycemic clamp technique was originally described by DeFronzo *et al*.[13] During this experiment, rats were allowed to move freely in a cardboard box.[14-17] The venous catheter was connected to pumps (STC-525, Terumo Co., Tokyo, Japan) for the infusion of either glucose, insulin, or L-NMMA. Insulin infusion at a rate of 3.0 $mU \cdot kg^{-1} \cdot min^{-1}$ was continued for 90 min. Arterial blood glucose concentration was determined at 10-min intervals, and a rate of 20% (w/v) glucose infusion was adjusted by feedback regulation to maintain the basal level (in normal rats) or a concentration of 8.0 mmol/l (in diabetic rats) over the 90-min period. As glucose infusion is considered equal to the glucose utilization in the body, the steady-state rate of glucose infusion obtained in the last 30 min of the euglycemic clamp can be used to evaluate the sensitivity of tissues to insulin.

For groups NGL and DGL, L-NMMA (1 $mg \cdot kg^{-1} \cdot min^{-1}$) was continuously infused throughout the 90-min euglycemic clamp. Arterial blood for insulin determination was sampled immediately before and 90 min after the start of the clamp study; blood samples were centrifuged at 4°C, and the plasma obtained was frozen at -80°C until analysis.

Analytical procedures

Blood glucose concentration was measured by the glucose oxidase method with a YSI 2300 glucose analyzer (Yellow Springs Instrument Inc., Yellow Springs, USA). Plasma insulin concentration was determined by radioimmunoassay (Insulin RIA-BEAD II, Dinabot Co., Tokyo, Japan).

Statistical analysis

The glucose disposal rate was obtained by adjusting the glucose infusion rate per body weight. The metabolic clearance rate of glucose (MCR) was then calculated by dividing the glucose disposal rate by the blood glucose concentration during the corresponding period of time. The average MCR obtained during the last 30 min of the clamp was regarded as an index of insulin action in insulin-sensitive tissues.

Values are expressed as the mean ± standard deviation (SD). Statistically significant differences among groups were determined by one-way factorial analysis of variance followed by Fisher's

protected least significant difference test. Statistical analyses were performed using the SPSS program, Version 8.0 (SPSS Inc., Chicago, USA). Statistical significance was set at 0.05.

RESULTS

Body weight and concentrations of blood glucose and plasma insulin

The body weight of diabetic rats immediately before the euglycemic clamp studies was significantly lower than that in normal rats (Table 2). Diabetic rats had significantly higher blood glucose and lower plasma insulin concentrations than normal rats (Table 2). Differences in the pre-clamp levels of blood glucose and plasma insulin among the NS, NG, and NGL groups and among the DS, DG, and DGL groups were not statistically significant.

Steady-state blood glucose (SSBG) and steady-state plasma insulin (SSPI) levels during the euglycemic clamp

According to the setting procedure of the euglycemic clamp for normal and diabetic rats, the SSBG levels in diabetic rats were significantly higher than those in normal rats, while the SSPI levels were not significantly different among the six groups of rats (Table 3).

Table 2 Body weight and concentrations of blood glucose and plasma insulin immediately before euglycemic clamp

	Body weight (g)	Glucose (mmol/l)	Insulin (pmol/l)
Control rats			
Saline	242 ± 9^{a}	3.8 ± 0.1^{c}	58 ± 20^{e}
GJG	240 ± 8^{a}	4.0 ± 0.1^{c}	48 ± 7^{e}
GJG + L-NMMA	245 ± 2^{a}	3.8 ± 0.2^{c}	53 ± 6^{e}
Diabetic rats			
Saline	216 ± 5^{b}	12.5 ± 0.7^{d}	26 ± 3^{f}
GJG	213 ± 7^{b}	12.4 ± 0.9^{d}	26 ± 4^{f}
GJG + L-NMMA	209 ± 3^{b}	11.8 ± 0.1^{d}	26 ± 3^{f}

Values represent the mean ± SD (n = 5). Different superscript letters in each item represent significant differences ($P < 0.05$, a vs. b, c vs. d, and e vs. f).

Table 3 Steady-state blood glucose and plasma insulin concentrations during euglycemic clamp at insulin infusion rate of 3.0 $mU \cdot kg^{-1} \cdot min^{-1}$

	SSBG (mmol/l)	SSPI (pmol/l)
Control rats		
Saline	4.1 ± 0.1^{a}	180 ± 22
GJG	4.0 ± 0.1^{a}	166 ± 23
GJG + L-NMMA	3.9 ± 0.1^{a}	176 ± 23
Diabetic rats		
Saline	7.9 ± 0.1^{b}	173 ± 20
GJG	7.5 ± 0.1^{b}	181 ± 20
GJG + L-NMMA	7.8 ± 0.1^{b}	166 ± 35

SSBG: steady-state blood glucose concentration; SSPI: steady-state plasma insulin concentration.
Values represent the mean ± SD (n = 5).
Different superscript letters represent significant difference ($P < 0.05$, a vs. b).

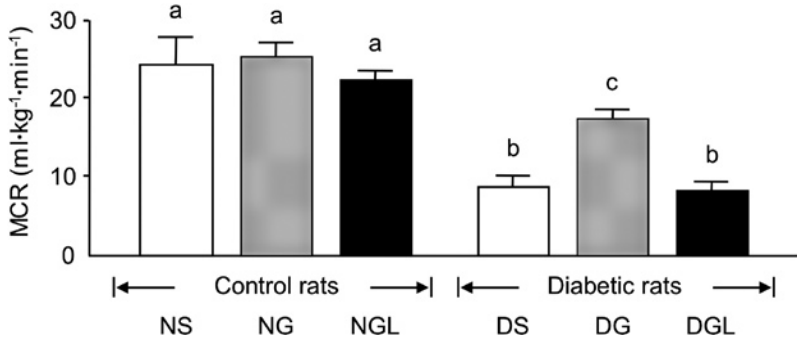

Fig. 1 MCRs during euglycemic clamp at insulin infusion rate of 3.0 mU·kg^{-1}·min^{-1}.
Data are the mean ± SD (n = 5). Different letters over the bars represent significant differences ($P < 0.001$, a vs. b; $P < 0.01$, a vs. c and b vs. c).

Metabolic clearance rate

As shown in Fig. 1, MCRs in the DS, DG, and DGL groups (8.7 ± 2.9, 18.2 ± 2.5, and 8.1 ± 1.8 ml·kg^{-1}·min^{-1}, respectively) were significantly lower than those in the NS, NG, and NGL groups (24.1 ± 4.5, 24.5 ± 3.1, and 22.2 ± 2.1 ml·kg^{-1}·min^{-1}, respectively). There was no significant difference among the NS, NG, and NGL groups. On the other hand, the MCR in the DG group was significantly higher than those in the DS and DGL groups. No significant difference between the DS and DGL groups was detected (Fig. 1).

DISCUSSION

STZ-induced diabetic animals are considered to be good models for human type 1 diabetes mellitus because, as occurs in humans, STZ destroys beta-cells in the pancreas. However, STZ-induced insulin deficiency also leads to insulin resistance,[18] and both the liver and skeletal muscle contribute to the worsening of insulin sensitivity in animals. An examination of the time-course changes in hepatic and muscular insulin action induced by STZ has shown that insulin resistance appears as early as one day after STZ injection.[19] In addition, the insulin suppression of hepatic glucose output was revealed to be impaired from day 3 after STZ injection.[19]

The insulinopenic state leads to increases in the number of hepatic insulin receptors in rats.[20] Furthermore, it has been reported that, although insulin binding to the plasma membrane of myocytes is increased in STZ-induced diabetic rats, intracellular signal transduction, the glucose transport system, and glucose metabolism are impaired.[21] Thus, these studies suggest that impairments in post-binding mechanisms in the liver and skeletal muscles are responsible for the defective insulin action in STZ diabetic rats.

MCRs obtained during euglycemic clamp studies, when an insulin infusion rate of 3.0 mU·kg^{-1}·min^{-1} raises plasma insulin levels to about 150 pmol/l, reflect postprandial insulin sensitivity in tissues such as the liver and skeletal muscle.[22,23] In the present study, insulin resistance in diabetic rats was evidenced by diminished MCRs during insulin infusion at 3.0 mU·kg^{-1}·min^{-1}. However, MCRs in diabetic rats administered with GJG (group DG) were significantly higher than those administered with saline (group DS). Even though no GJG effect was observed in normal rats, these results suggest that GJG may ameliorate whole-body glucose disposal in diabetic rats by improving the insulin action in insulin-sensitive tissues.

The NO synthase inhibitor L-NMMA is commonly used for the indirect evaluation of

NO-related insulin sensitivity or glucose uptake in peripheral tissues.[17,24,25] In this study, the amelioration of insulin resistance by GJG in diabetic rats was hampered by L-NMMA infusion. This outcome suggests that GJG may act through the effect of NO on the insulin action in insulin-sensitive tissues. Previous studies have shown that NO synthase activity in skeletal muscle is impaired during the progression of diabetes.[26,27] In addition, STZ-induced diabetes results in diminishing the blood flow in peripheral tissues.[28] Furthermore, it has been suggested that GJG might have a vasodilating effect on STZ diabetic rats via increased NO production.[8,11] However, we have shown in an earlier study that adenosine treatment, which elicits vasodilatation, does not bring about increases in insulin action in STZ-induced diabetic rats.[29] Accordingly, we hypothesize that GJG may improve insulin sensitivity via NO-related increased glucose uptake in peripheral tissues. This rationale would explain several studies in which NO was shown to increases glucose uptake by stimulating the translocation of the glucose transporter 4 to the cell surface in skeletal muscles.[30,31]

In contrast to hypoglycemic agents such as sulfonylureas,[32,33] GJG does not reduce blood glucose levels in healthy subjects and most likely affects insulin resistance only. Therefore, the results of both our previous study[12] and the present one suggest that GJG administration may not cause hypoglycemia, one of the most challenging side effects of hypoglycemic agents.

Our previous study has shown that daily GJG administrations for 1 month result in significantly reduced fasting blood glucose concentrations in diabetic patients.[34] In this study, plasma insulin levels of about 150 pmol/l achieved during the clamp experiments resemble the postprandial circulating insulin concentrations. Thus, GJG may also be clinically useful for the treatment of individuals with impaired glucose tolerance who show mild hyperglycemia only in the postprandial state. Nevertheless, confirming this inference calls for direct experimental evidence.

In conclusion, consecutive GJG administrations improved insulin resistance in STZ-induced diabetic rats, and the mechanism that mediated the effect of GJG may involve the NO pathway.

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