Role of herbal medicine (Kampo formulations) on the prevention and treatment of diabetes and diabetic complications

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We have studied the effects of herbal medicine (Kampo formulations) on the prevention and the treatment of diabetes and diabetic complication.

Effects of Kampo formulations on the treatment of diabetic neuropathy were investigated. The results obtained suggest that the combination of ten herbal medicines in Goshajinkigan(GJG) may have beneficial effects on metabolic and circulatory disturbances in nerves of diabetic patients. Therefore administration of GJG might be a useful approach for amelioration of the numbness and autonomic dysfunction associated with diabetic neuropathy.

Effects of Kampo formulations on in vivo insulin resistance were studied. 1. Animal experimental studies. The improvement of insulin resistance in STZ rats by the administration of GJG and Keishikajutsuboto(KJT) and cinnamon might be via NO pathway and due, at least in part, to correction in the abnormal early steps of insulin signaling pathway in skeletal muscle. 2. Clinical Studies. Effects of GJG on insulin resistance in patients with type 2 diabetes were investigated. HOMA-R was significantly decreased after GJG treatment (p=0.019). On the other hand, HOMA-R in the control group did not show significant difference. HOMA-R returned to the pre GJG treatment level 1 month after GJG discontinuation (P=0.018). The high-dose clamp resulted in a significantly increased insulin action (MCR levels) after GJG treatment.

These animal experimental and clinical studies suggest that GJG might be effective for improving insulin resistance in patients with type 2 diabetes.

In conclusion, Kampo formulations might be useful not only for the prevention and the treatment of diabetic complications but also effective for the prevention and treatment of type 2 diabetes.

Key words  Kampo formulations, diabetes, diabetic complications, Goshajinkigan, insulin action, euglycemic clamp.

Introduction

In Japan the number of diabetic patients has increased to 7.4 million and most diabetic patients are type 2 (non-insulin dependent). Decreased insulin secretion and insulin resistance play important roles in the occurrence and progression of type 2 diabetes.1)

On the other hand, approximately 3,500 patients / year lose their visual acuity because of diabetic retinopathy and nephropathy has been newly introduced in 13,900 patients / year with diabetic nephropathy in Japan in 2004. Additionally many diabetic patients are suffering from numbness, cold sensation and pains in the extremities derived from diabetic neuropathy. Therefore reduction of diabetic complications is one of the most important problems in the clinical field of diabetic patient care.2)

We have studied the effects of herbal medicine (Kampo formulations) on the prevention and the treatment of diabetes and diabetic complication for over 20 years. Here the results of our studies and the discussions are reviewed.

Effects of herbal medicine (Kampo formulations) on the treatment of diabetic neuropathy.

Management of diabetic neuropathy raises difficult problems. Many diabetic patients complain of numbness, cold sensation and pains in the lower extremities.3) A previous study from our laboratory showed that oral administration of Goshajinkigan (GJG; herbal medicine, Niu-Che-Sen-Qi-Wan) remarkably improved numbness and pains in the lower extremities due to diabetic neuropathy (general improvement ratio: 74.3%, utility: 71.6%).3,4)

In order to compare the clinical effects of GJG with mecobalamin (M) on diabetic neuropathy, a well-controlled comparative study was performed. The patients were randomly allocated to GJG (7.5 g/day) or M (1.5 mg/day) and observed at 4 week intervals for 12 weeks. Laboratory examinations were carried out before and after treatment.5)

Figure 1 shows the improvement rate of subjective symptoms. In group GJG, improvement rate of numbness of
the extremities was 74.2%, while it was 29.6% in group M (P<0.05). Among the eight symptoms (dizziness, constipation, hyperhidrosis, sexual hypofunction, impotence, dysuria, diarrhea and flushing) due to autonomic neuropathy, improvement rates in seven symptoms (except hyperhidrosis) were greater in the GJG than in the M group.

Laboratory examinations showed no significant changes after treatment in either group. The general improvement rate in GJG was 80.0%, but only 48.1% in the M group (P<0.01). Efficacy (P<0.05) and utility (P<0.05) of GJG were statistically higher than those of M.

It is evident that metabolic and vascular factors play important roles in the occurrence and development of diabetic neuropathy. Hyperglycemia of diabetes is associated with increased activity of aldose reductase (AR), accumulation of sorbitol in neuronal cells, and subsequent neuronal damage. GJG is thought to inhibit AR activity. In addition, GJG has vasodilating properties, thereby improving peripheral circulation and elevating skin temperature. Moreover, Aconite Tuber, a crude ingredient of GJG, exerts analgesic effects by secreting endogenous opioid, dynorphin in the spinal cord.

These results suggest that the combination of ten herbal medicines in GJG may have beneficial effects on metabolic and circulatory disturbances in nerves of diabetic patients. Therefore administration of GJG might be a useful approach for amelioration of the numbness and autonomic dysfunction associated with diabetic neuropathy.

Effects of herbal medicine (Kampo formulations) on the in vivo insulin resistance.


1) Effects of GJG on insulin resistance in streptozocin-induced diabetic rats.

As we already described GJG has been considered a useful medicine for the amelioration of complaints of the diabetic neuropathy. As for the mechanism of these effects, Suzuki et al. reported that GJG has vasodilating effects and improved peripheral circulatory disturbances in diabetic rats as a result of increase in nitric oxide (NO) production. Further, there has been evidence that the antinociceptive effect of GJG partly results from the peripheral action of increased production of the NO, which may be associated with increased rates of glucose transport and metabolism in skeletal muscle.

On the other hand previous studies from our laboratory showed that C-peptide has the capacity to increase whole body glucose utilization in streptozocin (STZ)-induced diabetic rats and the influence of C-peptide on glucose utilization may be mediated by NO.

These findings raised a question as to whether GJG could improve the insulin resistance in STZ diabetic rats and whether such improvement could be related to the NO pathway.

Consequently we examined the effects of GJG on insulin resistance in STZ (50mg/kg BW, iv) induced diabetic rats by means of euglycemic clamp procedure.

Diabetic and non-diabetic control rats were divided into the following three groups; a single dose of GJG (800mg/kg BW), saline (5ml/kg BW, p.o.) and GJG+N-monomethyl-L-arginine (L-NMMA 1mg/kg BW/min, an inhibitor of nitric oxide synthase-NOS). GJG consists of 10 medicinal plants (Table 1). A single dose of approximately 800 mg/kg BW, 10-fold higher than that used in humans, was dissolved in 5ml of saline and was given orally during the euglycemic clamp procedure. Three days after STZ intravenous injection the rats were anesthetized with sodium pentobarbital (50mg/kg BW). Thereafter, a middle ventral incision was made in the neck and the right jugular vein and left carotid artery were cannulated with Silascon SH tubing (Osaka, Japan). The catheters were tunneled subcutaneously to the dorsal region of the neck and flushed with 300 µl of saline containing heparin (40 U/ml) and 500µl of sodium penicillin G (10,000 U/ml). The catheters were then filled with a viscous solution of 50% polyvinylpyrrolidone (PVP) and capped with a piece of polyethylene tubing melted and sealed at one end. After surgery, the rats were kept at the same preoperative conditions. The food intake was recovered 2-3 days after surgery.
One week after surgery, each rat was submitted to a two-step hyperinsulinemic euglycemic clamp procedure in the conscious condition after an overnight fast to assess the whole-body insulin action. During the clamp study, jugular and carotid catheters were used for blood sampling and insulin and/or glucose infusion, respectively. The glucose infusion rate (GIR) in mg/kg BW/min was calculated every 10 min during the clamp study period. The means of GIR values during the euglycemic clamp procedure were regarded as an index of the whole body insulin action.

The metabolic clearance rate for glucose (MCR, ml/kg BW/min) was then obtained from GIR by dividing it with the corresponding blood glucose concentration. The MCRs were regarded as indices of insulin action in peripheral tissues. MCRs before and after GIG administration during the clamp are summarized in Table 2. The basal MCR of the diabetic rats were very low, approximately 32% of the control rats (P<0.05). The steady-state MCR of controls did not differ significantly from the basal MCR (Table 2 and Fig. 2). However, among diabetic rats the steady-state MCR was significantly higher (P<0.01) than basal MCR only in GIG administered diabetic group. Following medication, probably under the influence of stressor, the MCR fell in all groups for a while, returning to the basal levels approximately 60 min after medication (Fig. 2 A, B). There was no significant difference in ΔAUC (area under the curve) among control groups (Fig. 2 B). However, the ΔAUC of MCR in GIG administered diabetic rats was significantly higher (P<0.01) than those of saline administered diabetic and combined GIG and L-NMMA-administered diabetic rats (Fig. 2 D).

These results suggest that a single dose administration of

Table 2 Blood glucose and plasma insulin concentration and MCR before and after GIG administration during the euglycemic clamp.

<table>
<thead>
<tr>
<th>Clamp time (min)</th>
<th>Glucose (mmol/l)</th>
<th>Insulin (pmol/l)</th>
<th>MCR (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>210</td>
<td>90</td>
<td>210</td>
</tr>
<tr>
<td>Non-diabetic control rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline (5)</td>
<td>42±0.1</td>
<td>4.2±0.2</td>
<td>210±16</td>
</tr>
<tr>
<td>GIG (5)</td>
<td>4.4±0.2</td>
<td>4.5±0.1</td>
<td>217±28</td>
</tr>
<tr>
<td>GIG +l-NMMA (5)</td>
<td>4.2±0.1</td>
<td>4.1±0.1</td>
<td>208±17</td>
</tr>
<tr>
<td>Diabetic rats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline (5)</td>
<td>8.1±0.1×,†,‡,§</td>
<td>8.2±0.1×,†,‡,§</td>
<td>209±26</td>
</tr>
<tr>
<td>GIG (5)</td>
<td>8.4±0.1×,†,‡,§</td>
<td>8.0±0.3×,†,‡,§</td>
<td>220±45</td>
</tr>
<tr>
<td>GIG +l-NMMA (5)</td>
<td>8.5±0.2×,†,‡,§</td>
<td>8.2±0.2×,†,‡,§</td>
<td>210±7</td>
</tr>
</tbody>
</table>

The number of rats is shown in parenthesis. Values represent means ± SE.

* Significantly different from saline-administered control rats (P < 0.05)
† Significantly different from GIG-administered control rats (P < 0.05)
‡ Significantly different from the GIG +l-NMMA-administered control rats (P < 0.05)
§ Significantly different from saline and GIG +l-NMMA-administered diabetic rats (P < 0.05)


Fig. 2 Glucose MCR for the euglycemic clamp at a 3.0 mU kg⁻¹ min⁻¹ insulin infusion rate before and after the medicine administration. A, control rats; C, diabetic rats. The histograms represent the area under the curve (ΔAUC) of MCR (from 150 to 210 min), and are shown on the right side of the corresponding curve of MCR (B and D, for control and diabetic rats, respectively). ○, saline-administered rats; ●, GIG-administered rats; □, GIG +l-NMMA-administered rats. Data are means ± SE (n=5). NG and DG MCR compared to their respective mean bMCR (*P < 0.05, **P < 0.01); NS and DS MCR compared to their respective mean bMCR (†P < 0.05); NLG and DLG MCR compared to their respective bMCR (‡P < 0.05). Different characters represent significantly different values (P < 0.01) for the ΔAUCs.

GIG can improve the glucose utilization and insulin resistance in STZ diabetic rats, probably via the NO pathway.\(^3\)

2) GIG corrects abnormal insulin signaling.

Aims of the next study were as follows; 1) To investigate the long-term effects of insulin injection combined with GIG administration on the insulin sensitivity in STZ-induced diabetic rats. 2) To determine that GIG treatment potentiates the insulin action. 3) To analyze the molecular effects of combined GIG administration on the early steps of the insulin-signaling pathway in skeletal muscle.

Rats were randomized into five subgroups: (1) saline treated control, (2) GIG treated control, (3) 2-unit insulin + saline treated diabetic, (4) saline + GIG treated diabetic and (5) 2-unit insulin + GIG treated diabetic groups. After seven days of treatment, euglycemic clamp experiment at an insulin infusion rate of 6 mU/kg BW/min was performed in overnight fasted conscious rats (Fig. 3).

The expression of the insulin-signaling proteins in skeletal muscle was measured using the Western blotting method. Briefly, frozen samples were homogenized using a Polytron homogenizer, following which the homogenates were maintained at the ice temperature and centrifuged at 38,000 rpm at 4 °C for 1h. Supernatant proteins (40mg) of each sample were size-fractionated by SDS-PAGE and then transferred to PVDF membranes. The membranes were then incubated overnight with anti-insulin receptor (IR)-β and anti-insulin receptor substrate-1 (IRS-1) antibodies at 4 °C. Bound antibodies were detected by incubation with goat anti-rabbit IgG for 1h at room temperature. After washing, blotted proteins were visualized using the enhanced chemiluminescence detection system (ECL plus, Amersham). Quantification of the band intensity on the hyperfilm was performed using the public domain NIH image software.

The supernatants containing equal amounts of protein (1 mg/ml for each tube) were incubated overnight at 4 °C with anti-IR-β 85mg/ml or anti-IRS-1 85mg/ml, and then with 20μl of protein A agarose beads at 4 °C for 4h. The immune complexes were washed according to the procedure described by others. Samples were re-suspended in treatment buffer with β-mercaptoethanol and boiled for 5 min. Phosphorylated proteins were separated by SDS-PAGE and the membranes containing bound proteins were incubated with anti-phosphotyrosine antibody. Phosphorylated protein were visualized by the same method described above.

MCRs in diabetic rats were significantly lower compared with that in the controls. Combined insulin and GIG treatment and GIG administration significantly improve (P<0.01) MCRs in diabetic rats (Fig. 4). Insulin tolerance test shows that GIG administration significantly potentiates the impaired insulin action in diabetic rats (Fig. 5). These results agreed with the above data in which GIG administration improved the insulin-regulated glucose uptake in STZ-diabetic rats.

The increased IR-β protein content in skeletal muscle of diabetic rats was not affected by insulin combined with GIG administration (Fig. 6 A). However the combination of insulin and GIG significantly (P<0.05) improved the decreased IRS-1 protein content (Fig. 7 A).\(^{11}\)

![Fig. 3 Time-table for the experimental procedures. The number of rats is shown in parentheses. Surgical procedures (#) were performed 3 days after STZ treatment.](Qin B et al., eCAM 1 (3): 269-276, 2004)

![Fig. 4 MCRs during the euglycemic clamp procedure in normal and diabetic rats (insulin + saline; insulin + GIG; saline + GIG). Data are expressed as the means ± SE for six rats in each group. *P < 0.01 vs insulin + GIG - treated and saline + GIG - treated diabetic. **P < 0.001 vs all diabetic groups.](Qin B et al., eCAM 1 (3): 269-276, 2004)
The tyrosine phosphorylation level of IR-β was determined by immunoblotting the IR-β antibody immunoprecipitates with the phosphotyrosine antibody. As shown in Fig. 6 B, the tyrosine phosphorylation level of IR-β in skeletal muscle of STZ-diabetes was significantly increased when compared with control (140% of control, P<0.05). The overexpressed tyrosine phosphorylation of IR-β was corrected by GJG treatment. The same trend was found in IRS-1 tyrosine phosphorylation. As shown in Fig. 7 B, the abnormal increases in IRS-1 tyrosine phosphorylation induced by STZ (137% of control, P<0.05) was just about recovered by GJG treatment.  

These results suggest that the improvement of insulin resistance in STZ rats by the administration of GJG might be due, at least in part, to correction in the abnormal early steps of insulin signaling pathway in skeletal muscle.  

3) Effects of Keishikajutsubuto(Guizhiashutang) on the decreased insulin action in STZ-induced diabetic rats.

Keishikajutsubuto(KJT), one of the traditional herbal medicines, consists of seven crude herbs (Table 3) and

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Fig. 5 Insulin tolerance test: blood glucose levels of the insulin + GJG-treated diabetic group tended to be lower than insulin-treated diabetic group at the 0 min time point, but not significantly. At the 120 min time point, the average blood glucose of the insulin + GJG-treated group was significantly lower than the only insulin injection group. Data are expressed as the means ± SE for six rats in each group. *P < 0.05. (Qin B et al., eCAM 1 (3): 269-276, 2004)

Fig. 6 IR-β protein content (A) and tyrosine phosphorylation levels (B) in the gastrocnemius muscle. IP, Immunoprecipitation; IB, Immunoblotting: Ty, Phosphotyrosine. Data are expressed as the means ± SE for five rats in each group. *P < 0.05 vs insulin + saline-treated and insulin + GJG-treated diabetic. **P < 0.05 vs controls and insulin + GJG-treated diabetic. (Qin B et al., eCAM 1 (3): 269-276, 2004)

Fig. 7 IRS-1 protein content (A) and tyrosine phosphorylation levels (B) in gastrocnemius muscle. IP, Immunoprecipitation; IB Immuno-blotting: Ty, Phosphotyrosine. Data are expressed as the means ± SE for five rats in each group. *P < 0.05 vs insulin + saline-treated diabetic. **P < 0.001 vs all diabetic groups. (Qin B et al., eCAM 1 (3): 269-276, 2004)
Table 3 Composition of KJT

<table>
<thead>
<tr>
<th>Component</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cinnamomi Cortex</td>
<td>20.51</td>
</tr>
<tr>
<td>Paeoniae Radix</td>
<td>20.51</td>
</tr>
<tr>
<td>Glycyrrhiza Radix</td>
<td>10.25</td>
</tr>
<tr>
<td>Zizyphi Fructus</td>
<td>20.51</td>
</tr>
<tr>
<td>Zingiberis Rhizoma</td>
<td>5.12</td>
</tr>
<tr>
<td>Atractyloidis Lanceae</td>
<td>20.51</td>
</tr>
<tr>
<td>Aconiti Tuber</td>
<td>2.56</td>
</tr>
</tbody>
</table>

The KJT used in this study was an unprepared bulk powder. 
(Qin B et al., Life Sciences. 73: 2687-2701, 2003)

could induce vasodilatation, analgesics, and has anti-inflammatory action.19,20 KJT has been used for the treatment of various diseases, such as neuropathy, arthralgia and rheumatic arthritis.19 Basic experimental studies showed that KJT inhibited aldose reductase in rat lens and sorbitol accumulation in human erythrocytes.20

On the other hand NO is an important endogenous vasodilator that mediates various physiological functions. Decreased nitric oxide synthase (NOS) activity plays some important roles on the occurrence and progression of diabetic complications such as retinopathy, nephropathy and neuropathy.21 Additionally Chandra et al.22 reported that NO prevents AR activation and sorbitol accumulation during diabetes. The above findings raised us a question: may the pharmacological mechanism of KJT be related to NO? Steinberg et al.23 indicated that NO was involved in the regulation of skeletal muscle hemodynamics and glucose utilization through vasodilatation associated with insulin. Consequently we investigated whether decreased insulin action in STZ diabetic rats could be improved by KJT treatment using the euglycemic clamp technique.11,12,15 Based on preliminary results we also analyzed whether the expected improvement in insulin action in STZ diabetic rats by KJT administration was mediated enhanced NO production. Further we examined the effects of KJT on the molecular mechanism of insulin signaling: IR-β, IRS-1, phosphatidylinositol 3 kinase (PI-3-kinase), and glucose transporter 4 (GLUT4) in the skeletal muscle.16,17

A single dose of KJT 800mg/kg BW, approximately 10-fold higher than that used in humans, dissolved in 5ml/kg saline, was administered orally through a gastric tube.

Euglycemic clamp (insulin infusion rate: 3 and 30mU/kg BW/min) was performed in awaked rats.

At low-dose(insulin infusion rate : 3mU/kg BW/min), the decreased MCR in diabetic rats were significantly improved by a single and 7 days administration of KJT (Table 4, Fig. 8).15

During high-dose insulin infusion (insulin infusion rate: 30mU/kg BW/min), the MCR was increased in 7 days KJT treated diabetic rats compared with saline treated diabetic rats, while these changes were not observed after a single KJT administration (Table 4).15 The increased MCR induced by the 7 days KJT administration was blocked by L-NMMA (Table 4, Fig. 8). However no further additive effects were noticed in KJT and sodium nitroprusside (SNP:NO donor) administration.15

IR-β protein increase and decreased IRS-1 protein expression in diabetic rats were significantly improved by KJT administration (Fig. 9, 10).15 The increased tyrosine 3-phosphorylation levels of IR-β, IRS-1 and IRS-1 associated with PI-3 kinase were significantly inhibited in KJT admin-

Table 4 Metabolic clearance rate (MCR) of glucose for the last 30 min during the euglycemic clamp procedure at the low-dose (0-90 min) and the high-dose (90-180 min) insulin infusion

<table>
<thead>
<tr>
<th>Group</th>
<th>MCRs for glucose (ml/kg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low-dose clamp</td>
</tr>
<tr>
<td><strong>Acute administration effect</strong></td>
<td></td>
</tr>
<tr>
<td>Non-diabetic</td>
<td></td>
</tr>
<tr>
<td>Saline treatment (7)</td>
<td>15.1 ± 0.6</td>
</tr>
<tr>
<td>KJT treatment (6)</td>
<td>14.9 ± 0.7</td>
</tr>
<tr>
<td>Diabetic</td>
<td></td>
</tr>
<tr>
<td>Saline treatment (7)</td>
<td>6.7 ± 0.6²</td>
</tr>
<tr>
<td>KJT treatment (7)</td>
<td>12.3 ± 1.2²</td>
</tr>
<tr>
<td><strong>7-days administration effect</strong></td>
<td></td>
</tr>
<tr>
<td>Non-diabetic</td>
<td></td>
</tr>
<tr>
<td>Saline treatment (6)</td>
<td>15.8 ± 0.9</td>
</tr>
<tr>
<td>Diabetic</td>
<td></td>
</tr>
<tr>
<td>Saline treatment (6)</td>
<td>6.3 ± 0.5²</td>
</tr>
<tr>
<td>KJT treatment (6)</td>
<td>13.9 ± 1.0²</td>
</tr>
<tr>
<td>KJT treatment + L-NMMA (5)</td>
<td>6.9 ± 1.2²</td>
</tr>
<tr>
<td>Saline treatment + SNP (5)</td>
<td>14.0 ± 1.0</td>
</tr>
<tr>
<td>KJT treatment + SNP (5)</td>
<td>13.7 ± 1.2</td>
</tr>
</tbody>
</table>

Values are means ± S.E. The number of rats in each group is shown in parentheses.

²Indicates a P < 0.001 vs. non-diabetic rats.

²Indicates a P < 0.001 vs. saline treated diabetics.

²Indicates a P < 0.05 vs. saline treated diabetics.

(Qin B et al., Life Sciences 73: 2687-2701, 2003)

Fig. 8 7-day administration effect: MCRs during the euglycemic clamp procedure in normal control and diabetes at the low-dose insulin infusion in the treatment with saline (normal and diabetes, n=6, respectively), and KJT (n=6), KJT+L-NMMA (n=5), KJT + SNP (n=5), and SNP (n=5) treated diabetic rat, respectively. Data are expressed as the means ± S.E. *P < 0.001 vs. KJT treated diabetic and normal control. 
(Qin B et al., Life Sciences 73: 2687-2701, 2003)
Fig. 9 Effect of 7-day administration of KJT on IR-β protein content (A) and tyrosine phosphorylation (B) in rat gastrocnemius muscle. Immunoprecipitation: IP; Immunoblotting: IB; Phosphotyrosine: PT. Data are expressed as the means ± S.E. for 5 rats, respectively. *P < 0.05 vs. controls; **P < 0.001 vs. controls and KJT diabetic groups.
(Qin B et al., Life Sciences 73: 2687-2701, 2003)

Fig. 10 Effect of 7-day administration of KJT on IRS-1 protein content (A) and tyrosine phosphorylation (B) in rat gastrocnemius muscle. Immunoprecipitation: IP; Immunoblotting: IB; Phosphotyrosine: PT. Data are expressed as the means ± S.E. for 5 rats, respectively. *P < 0.05 vs. saline diabetic group. **P < 0.001 vs. controls.
(Qin B et al., Life Sciences 73: 2687-2701, 2003)

Fig. 11 Effect of 7-day administration of KJT on PI 3-kinase protein content (A) and the association of PI 3-kinase with IRS-1 (B) in rat gastrocnemius muscle. Immunoprecipitation: IP; Immunoblotting: IB. Data are expressed as the means ± S.E. for 5 rats, respectively. *P < 0.05 vs. saline diabetic group; **P < 0.001 vs. controls.
(Qin B et al., Life Sciences 73: 2687-2701, 2003)

Fig. 12 Effect of 7-day administration of KJT on GLUT-4 protein content in rat gastrocnemius muscle. Data are expressed as the means ± S.E. for 5 rats. *P < 0.001 vs. saline and KJT treated diabetic groups.
(Qin B et al., Life Sciences 73: 2687-2701, 2003)

istered diabetic rats (Fig. 9, 10, 11). KJT had no effect on the GLUT 4 protein level (Fig. 12).)

These results suggest that improvement of decreased insulin action observed in STZ diabetic rats by KJT administration might be, at least in part, to enhanced insulin signaling, and subsequent ameliorated production of NO. Therefore this study supports the clinical usefulness of KJT for the treatment of diabetic neuropathy. (5)

4) Cinnamon extract potentiates in vivo insulin action via enhancing insulin signaling in rats.

Cinnamon, one of the 7 constituents of KJT, has been mentioned in Chinese medical texts as long as 2,000 years ago. It has been reported that the cinnamon extract has vasodilative, anti-thrombotic, anti-spasmodic, anti-ulcerous, and anti-allergic action. In vitro studies revealed that the cinnamon extract mimics the effect of insulin, which potentiates insulin action in isolated adipocytes. It is believed that the methylhydroxycalcone polymer (MHCP, extracted from cinnamon) is responsible for the above effects. MHCP may be useful in the treatment of insulin resistance via increasing glucose utilization in cells. However, to our knowledge, up till now the effect of the cinnamon extract on insulin action has not been demonstrated in vivo studies.

Aims of the next study were as follows: 1) To investigate whether insulin action was really improved by the cinnamon extract treatment in Wistar rats using the euglycemic clamp technique. 2) To determine whether the cinnamon extract could enhance insulin signaling mechanisms in skeletal muscle.

We performed two-step sequential euglycemic clamp procedure (low-dose clamp: insulin infusion rate 3mU/kg BW/min, 0-90 min; high-dose clamp: 30mU/kg BW/min, 90-180min).

GIrS for the euglycemic clamp procedure at the low-dose (3.0mU/kg BW/min) and the high-dose (30.0 mU/kg BW/min) insulin infusion rates for animals treated with saline, 30 and 300mg/kg BW cinnamon extract are illustrated in Fig. 13. The average GIrS for the last 30 min during low- and high-dose clamp are shown in Fig. 14.
Three weeks after the cinnamon extract administration (30 and 300 mg/kg BW: C30 and C300), cinnamon extract treated rats showed a significantly higher GIR at low-dose clamp. At high-dose clamp the GIR in C300 was increased 17% over controls.\textsuperscript{27}

No significant difference in the protein content of IR-β, IRS-1 and PI3-kinase was detected (Fig. 15A, 16A, 17A, respectively).

The tyrosine phosphorylation level of IR-β in skeletal muscle of the cinnamon extract administered animals was significantly increased when compared with controls (P<0.05) (Fig. 15B).\textsuperscript{27} The same tendency was found for the IRS-1 tyrosine phosphorylation (133% of controls, P<0.01), as illustrated in Fig. 16B. The IRS-1/PI3-kinase association level was determined by immunoblotting the PI3-kinase antibody immunoprecipitates with the IRS-1 antibody. The IRS-1/PI3-kinase association had a significant increase in the skeletal muscles of cinnamon extract treated animals (P<0.01) (Fig. 17B).\textsuperscript{27}

These results suggest that the oral administration of cinnamon extract would improve in vivo insulin-regulated whole-body glucose utilization in a dose-dependent fashion in rats, at least in part, through enhancing the insulin signal pathway in skeletal muscle.\textsuperscript{27}

2. Clinical studies

Effects of GJG on insulin resistance in patients with type 2 diabetes.

As we already described in this monograph, GJG could improve glucose utilization and insulin resistance in...
STZ-induced diabetic rats and this improvement might be due, at least in part, to correction in the abnormal early steps of insulin signal pathway in skeletal muscle.\(^{(14,18)}\)

However up till now it has not been assessed whether GIGJ has similar effects in humans.\(^{(20)}\) Consequently in order to investigate the effects of GIGJ administration on insulin sensitivity in type 2 diabetic patients, the following studies were performed using two methods: the homeostasis model assessment of insulin resistance (HOMA-R) and the euglycemic clamp technique.

1) HOMA-R study.

Daily oral administration of GIG 7.5g/day was carried out for 1 month in 71 type 2 diabetic patients. HOMA-Rs were calculated before and after 1 month of GIGJ treatment and compared with those of 44 control type 2 diabetic patients who were matched in terms of sex, age, BMI and HbA1c levels with the GIGJ group.

HOMA-R was calculated using fasting blood glucose (FBG) and fasting immuoreactive insulin (FIRI) values and was used for the evaluation of insulin resistance using the following formula\(^{(20)}\): HOMA-R=FIRI (µU/ml) × FBG (mg/dl)/405.

2) GIRs and MCRs determined by the euglycemic clamp procedure.

Insulin action (MCR) in 8 patients with type 2 diabetic patients was estimated two-sequential euglycemic clamp (low-dose clamp: insulin infusion rate: 40 mU/m²/min; insulin sensitivity, high dose clamp : 400 mU/m²/min; insulin responsiveness) before and after 1 month GIGJ treatment (7.5 g/day).\(^{(30)}\)

During the low-dose clamp there was no significant difference in MCR between pre-and post-GIGJ treatment (4.1

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Table 5 Changes in BMI and blood biochemical parameters in the control group and the GIG treatment group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Before</th>
<th>After</th>
<th>P-value</th>
<th>n</th>
<th>GIG treatment Before</th>
<th>After</th>
<th>P-value</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>23.2 ± 0.4</td>
<td>23.2 ± 0.4</td>
<td>0.124</td>
<td>444</td>
<td>24.2 ± 0.4</td>
<td>24.2 ± 0.4</td>
<td>0.295</td>
<td>711</td>
</tr>
<tr>
<td>FBG (mg/dl)</td>
<td>180 ± 6</td>
<td>177 ± 6</td>
<td>0.609</td>
<td>44</td>
<td>180 ± 5</td>
<td>167 ± 5</td>
<td>0.005</td>
<td>71</td>
</tr>
<tr>
<td>FIRI (µU/ml)</td>
<td>9.9 ± 0.6</td>
<td>9.9 ± 0.6</td>
<td>0.992</td>
<td>44</td>
<td>11.1 ± 0.7</td>
<td>10.4 ± 0.7</td>
<td>0.145</td>
<td>71</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.8 ± 0.2</td>
<td>7.8 ± 0.2</td>
<td>0.677</td>
<td>44</td>
<td>8.0 ± 0.2</td>
<td>8.0 ± 0.2</td>
<td>0.719</td>
<td>71</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>211 ± 4</td>
<td>213 ± 5</td>
<td>0.718</td>
<td>44</td>
<td>212 ± 4</td>
<td>206 ± 3</td>
<td>0.016</td>
<td>70</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>55 ± 2</td>
<td>54 ± 2</td>
<td>0.430</td>
<td>44</td>
<td>45 ± 1</td>
<td>45 ± 1</td>
<td>0.149</td>
<td>70</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>140 ± 18</td>
<td>137 ± 20</td>
<td>0.770</td>
<td>44</td>
<td>181 ± 18</td>
<td>161 ± 15</td>
<td>0.047</td>
<td>70</td>
</tr>
</tbody>
</table>

Values are means ± S.E. GIGJ: Goshajinkigan; Before: before 1-month period; After: after 1-month period; Pre-GIGJ: pre-GIGJ treatment; Post-GIGJ: post-GIGJ treatment; BMI: body mass index; FBG: fasting blood glucose; FIRI: fasting immuoreactive inslin; TC: total cholesterol; HDL-C: high density lipoprotein-cholesterol; TG: triglyceride.

± 1.2 ml/kg/min and 4.5 ± 0.8 ml/kg/min, respectively (P=0.69). However the high-dose clamp resulted in significantly increased MCR levels after GIG treatment (from 7.9 ± 0.8 to 9.1 ± 0.8 ml/kg/min, P<0.046) (Fig. 20).28)

These results indicate that GIG administration might be useful for enhancing the deteriorated insulin action in patients with type 2 diabetes.28)

Previous study from our laboratory3-5) showed that GIG could ameliorate subjective symptoms of the patients with diabetic neuropathy and is effective for the prevention of occurrence and progression of diabetic neuropathy.

From recent animal experiments14-15) and clinical studies28) we could demonstrate that GIG might be effective for improving insulin resistance in the patients with type 2 diabetes.

In conclusion, Chinese herbal medicines (Kampo formulations) might be useful not only for the prevention and the treatment of diabetic complications but might also be effective for the prevention and treatment of type 2 diabetes.

Acknowledgement

This research was supported by Longevity Science Research Grants from the Ministry of Health and Labor of Japan (93A1106, H9-025, H13-009), Grant in Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (11670066) and grants from the Kampo Medicine Institute of Japan (2001, 2003).

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**Japanese abstract**

我々は糖尿病および糖食病合併症に対する漢方薬の有用性について20年以上動物実験的、臨床的に検討を加えてきたので紹介する。

1. 糖尿病中性障害に対する牛車腎気丸の効果

糖尿病中性障害に対する牛車腎気丸の効果をメタバロミンと比較した。しまいに対し、前者の改善率が後者より有意に大であった。この成績は牛車腎気丸が糖尿病中性障害の治療に有用であることを示唆している。

2. インスリン抵抗性に対する漢方薬の効果

1) 動物実験では、STZ糖尿病ラットに対し、牛車腎気丸、桂枝加朮附湯およびその構成薬で牛皮を投与したところ、インスリン感受性は有意に改善した。また、そのメカニズムとして一酸化窒素（NO）とインスリンシグナル伝達系の関与が示唆された。

2) 臨床研究では、2型糖尿病患者に対し、牛車腎気丸を7.5 g/日を1ヶ月間投与したところ、HOMA-R（インスリン抵抗性）は有意に改善した。正常血糖クランプ法でも検討を加えたが、高インスリン注入量クランプにて有意に増大した。この成績は牛車腎気丸が2型糖尿病患者のインスリン抵抗性を改善する可能性を示唆している。

以上、我々の検討成績は漢方薬が糖尿病合併症の予防・治療だけでなく、糖尿病の予防・治療にも有用である可能性を示唆している。

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