Review

Chinese Medicine, Jiang-Tang-Ke-Li, Improves Insulin Resistance by Modulating Muscle Fiber Composition and Muscle Tumor Necrosis Factor- α in Fructose-Fed Rats

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Insulin resistance and hyperinsulinemia are common findings in patients with essential hypertension. These impairments in glucose metabolism are commonly associated with diabetes mellitus, hypertension, and dyslipidemia, which are high risk factors of cardiovascular diseases, and recent evidence indicates that they may play a role in the development of coronary artery disease. The aim of this study was to determine the effect of Jiang-Tang-Ke-Li (JTKL), a traditional Chinese medicine used to treat diabetes mellitus in China, on insulin resistance and hypertension in fructose-fed rats (FFR). Systolic blood pressures in the FFR groups were significantly higher than that in the control group, although JTKL had no effect on systolic blood pressure for the last 2 weeks of treatment with the medicine. The average rate of glucose infusion during a glucose clamp, as an index of insulin sensitivity (M value), was significantly lower in the FFR than in the control rats, and treatment with JTKL for 2 weeks significantly increased the M value to that of the control. Treatment with *Panax ginseng* (PG), a component of JTKL, for 2 weeks also significantly increased the M value of FFR to the control level. The composite ratio of type I fibers in soleus muscle decreased significantly in the FFR compared to that in the control, and treatment with JTKL led to recovery of the composite ratio of type I fibers to the same level as that of the control group. The M value showed a significant positive correlation with the composite ratio of type I fibers and a significant negative correlation with the composite ratio of type II fibers. Tumor necrosis factor (TNF)- α levels were significantly higher in the soleus and extensor digitorum longus (EDL) muscles of the FFR than in those of the control rats. Treatment with JTKL for 2 weeks significantly lowered TNF- α levels to the control levels. M values showed a significant negative correlation with TNF- α in both the soleus and EDL muscles. The results suggest that the Chinese medicine JTKL, which contains PG as one of its valid components, improves insulin resistance by modulating muscle fiber composition and TNF- α in skeletal muscles in hypertensive and insulin-resistant FFR.

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Key Words: insulin resistance, muscle fiber composition, tumor necrosis factor (TNF)- α , Jiang-Tang-Ke-Li (JTKL), fructose-fed rats

Introduction

Insulin resistance and accompanying hyperinsulinemia have

recently been reported to play important roles in the onset and persistence of essential hypertension, dyslipidemia, and arteriosclerosis (1–3). Insulin resistance has also been reported in several animal models of hypertension, including the

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Table 1. The Ratio of Fiber Composition in Soleus Muscle

	Group (%)		
	Control	FFR	FFR + JTKL
Type I	81.7 ± 1.5	75.0 ± 1.7*	78.8 ± 1.1
Type II	18.3 ± 1.5	25.1 ± 1.7*	21.2 ± 1.1
Type IIa	18.2 ± 1.3	24.1 ± 1.6*	20.1 ± 1.5
Type IIb	0.3 ± 1.3	1.2 ± 1.7	1.1 ± 0.4

FFR + JTKL, fructose-fed rats (FFR) treated with Chinese medicine, Jing-Tang-Ke-Li (JTKL). Values are mean ± SEM.

* $p < 0.01$ vs. control and FFR + JTKL.

spontaneously hypertensive rat and the fructose hypertensive rat (4). Fructose-fed rats (FFR) show an acquired form of hypertension, in which the rise in blood pressure is not genetically determined but is diet-induced (5). Although it has been speculated that the rise in blood pressure is secondary to the development of insulin resistance and hyperinsulinemia, the precise mechanism of insulin resistance in FFR remains to be clarified.

The importance of skeletal muscle in insulin-mediated glucose metabolism has been established by a number of investigators (6–8). Euglycemic hyperinsulinemic glucose clamp studies have demonstrated that skeletal muscle accounts for more than 80% of glucose disposal under hyperinsulinemic conditions in humans (8). Several animal studies have demonstrated that substantial differences in insulin-mediated glucose uptake exist between muscle groups, and that these differences may be related to muscle fiber composition (9–11). Moreover, it has been shown that insulin action is greater in red type I (slow twitch, oxidative) and type IIa (fast twitch, oxidative/glycolytic) fiber types than in the white type IIb (fast twitch, glycolytic) fiber type (11). We previously reported that the composite ratio of type I fibers in the soleus muscle is lower in FFR than in controls (12). This suggests that changes in muscle fiber composition may be an important mechanism in insulin resistance in FFR.

Tumor necrosis factor (TNF)- α has long been regarded as a pre-inflammatory cytokine that is released from macrophages or monocytes and kills tumor cells or microorganisms. Recent studies have suggested that TNF- α may also play a role in the development of insulin resistance, especially in patients with type 2 diabetes mellitus or obesity and in animal models with genetical obesity or diet-induced obesity (13–15). It has been suggested that adipose tissue-derived TNF- α may suppress the glucose uptake by insulin through the inhibition of insulin receptor tyrosine kinase activity (16). Recently, it has been reported that skeletal muscles themselves also express TNF- α , and this expression would inhibit glucose uptake by skeletal muscles in an autocrine or paracrine fashion and cause insulin resistance (17). Another study has shown that adipocyte-derived TNF- α is not correlated with insulin action in aging rats (18). We previously reported that skeletal muscle TNF- α is linked to insulin resistance

Fig. 1. Comparison of M values between controls and FFR with or without PG.

tance and that angiotensin II may be one of the factors that regulate skeletal muscle TNF- α in FFR (19). Further studies are needed to clarify the correlation between TNF- α and insulin resistance in detail.

A number of Chinese medicines of botanical origin, such as Qing-Xin-Lian-Zi-Yin (QX), are thought to be effective for treating diabetes mellitus, especially type 2 diabetes mellitus. And although the concept of insulin resistance does not exist in traditional Chinese medicine, it has recently been reported that QX improved insulin-stimulated glucose uptake in peripheral tissues in streptozotocin-induced diabetic rats (20). However, the mechanism by which QX, a mixture of crude extracts of Chinese medicinal plants, improves insulin sensitivity is not yet understood. We previously reported that Tang-Shen-Jiao-Nang (TSJN) improved insulin sensitivity of FFR (21). On the other hand, while Jiang-Tang-Ke-Li (JTKL), a Chinese medicine for diabetes mellitus that is believed to lower blood glucose, has been used in China to treat patients with type 2 diabetes mellitus, the mechanisms by which it lowers blood glucose levels have not yet been clarified.

In this context, this study was designed to clarify the effects of JTKL on insulin resistance and hypertension and to try to determine the mechanisms by which JTKL improves insulin sensitivity of FFR.

FFR and JTKL

Basal Characteristics of FFR and Effect of JTKL

Six-week-old male Sprague-Dawley rats (Charles River Japan Inc., Yokohama, Japan) were used for the experiments. The care of the animals was in strict accordance with

Fig. 2. Correlations between M value and composition ratio of type I fiber (A), and type II fiber (B).

the guiding principles of the Physiological Society of Japan. Permission for the study was granted by the Ethics Committee on Animal Experiments, Sapporo Medical University. Prior to any manipulation, rats were fed either a standard rat chow containing 60% vegetable starch, 5% fat, and 24% protein (Oriental Yeast Co., Tokyo, Japan) as a control group or a fructose-rich chow containing 60% fructose, 5% fat, and 20% protein (Teklad, Madison, USA) for 6 weeks. The rats fed the fructose-rich chow were treated either with a vehicle (2.5% gum arabic solution) for the last 2 weeks (FFR, $n = 16$) or with 3.24 g/kg/day of JTKL (provided by Prof. Li Wen Rui, Beijing Hospital, P.R. China; FFR + JTKL, $n = 14$) for the last 2 weeks. Systolic blood pressure (SBP) was elevated significantly at 2 weeks after the start of the fructose-rich chow feeding, and this high level was maintained in FFR throughout the study period. SBP at 12 weeks of age in the FFR (143 ± 2 mmHg) was significantly higher than that in the control group (133 ± 2 mmHg), and treatment with JTKL for 2 weeks had no effect on the SBP of the FFR (142 ± 2 mmHg). There were no differences in heart rate or fasting blood glucose level among the three groups. The fasting insulin level was significantly ($p < 0.01$) higher in FFR (265 ± 57 pmol/l) than in control rats (120 ± 29 pmol/l), and JTKL lowered the fasting insulin level (213 ± 28 pmol/l), but the decrease was not significant (22).

Glucose Clamp Study

A euglycemic hyperinsulinemic glucose clamp (GC) was performed to evaluate insulin sensitivity, which was expressed as an M value as described previously (12). The M value was significantly ($p < 0.01$) lower in the FFR (11.5 ± 0.6 mg/kg/min) than in the control rats (15.5 ± 1.0 mg/kg/min). JTKL significantly ($p < 0.01$) improved the M value in the FFR + JTKL group (15.0 ± 0.7 mg/kg/min) compared to that in the FFR group (22). To further examine the effects of JTKL on insulin resistance, we evaluated the effects of

Panax ginseng (PG), a component of JTKL, on insulin sensitivity. Treatment with PG for 2 weeks significantly increased the M value of FFR to control levels (12.7 ± 1.1 , 15.3 ± 0.9 and 16.0 ± 1.0 mg/kg/min for FFR, control and FFR + PG, respectively, $p < 0.01$, Fig. 1).

Muscle Fiber Compositions

Muscle fiber compositions were determined by the adenosine-triphosphatase method as described previously (12). The composite ratio of type I fibers in the soleus muscle decreased significantly ($p < 0.01$) in the FFR ($75.0 \pm 1.7\%$) compared to that in the control group ($81.7 \pm 1.5\%$), and treatment with JTKL led to recovery of the composite ratio of type I fibers in the FFR + JTKL group ($78.8 \pm 1.1\%$) to approximately the same level as in the control group (Table 1). The composite ratio of type IIa fibers in the soleus muscle increased significantly in the FFR compared to that in the control rats, and JTKL led to recovery of the composite ratio of type IIa fibers in the FFR + JTKL group to the same level as in the control group. The M value was significantly correlated with the composite ratio of type I and type II fibers (for type I fibers, $r = 0.33$, $p < 0.05$, Fig. 2A; for type II fibers, $r = -0.33$, $p < 0.05$, Fig. 2B). No correlations were found between the M value and body weight, fasting blood glucose level or plasma insulin level (data not shown). Thus, treatment with JTKL not only improved insulin sensitivity but also led to recovery of the muscle fiber composite ratio in FFR.

TNF- α in Muscles

TNF- α was measured using a rat ELISA kit (Biosource International, Camarillo, USA) as described previously (22). There were no significant differences in either soleus muscle or extensor digitorum longus (EDL) muscle weights among the three groups (data not shown). As shown in Fig. 3, TNF-

Fig. 3. Correlations between M value and tumor necrosis factor (TNF)- α in (A) the soleus muscle and (B) the extensor digitorum longus (EDL) muscle.

α in the soleus muscle was significantly ($p < 0.001$) higher in the FFR (480 ± 46 pg/g wet tissue) than in the control rats (177 ± 34 pg/g wet tissue), and a significant difference was also seen between the TNF- α levels in EDL muscle of the FFR and control groups (206 ± 33 pg/g wet tissue and 570 ± 45 pg/g wet tissue in control rats and FFR, respectively; $p < 0.001$). JTKL significantly lowered TNF- α in both the soleus muscle (189 ± 22 pg/g wet tissue in the FFR + JTKL group, $p < 0.01$ compared to the FFR group) and EDL muscle (239 ± 92 pg/g wet tissue in the FFR + JTKL group, $p < 0.01$ compared to the FFR group). M values showed a significant negative correlation with TNF- α in both the soleus muscle ($r = -0.48$, $p < 0.01$, $n = 29$) and EDL muscles ($r = -0.65$, $p < 0.001$, $n = 29$) (Fig. 3).

Discussion

Insulin Resistance in FFR

The results of this study confirmed those of previous studies showing that feeding healthy rats a fructose-rich chow results in the development of insulin resistance and hypertension (23). It has been reported that hyperinsulinemia can stimulate activation of the sympathetic nervous and renin-angiotensin systems, cause an increase in renal sodium retention, and induce proliferation of vascular smooth muscle tissue, and that hyperinsulinemia may be involved in the development of hypertension (24). Insulin resistance and hyperinsulinemia were seen in the FFR in the present study. These results suggest that fructose feeding leads to insulin resistance and that compensatory hyperinsulinemia may result in hypertension. And although the precise mechanisms by which fructose-rich chow confers insulin resistance are still obscure, we have previously reported that the high levels of

free fatty acid in the serum of FFR (25) may play a role in this process.

Muscle Fiber Compositions

It has been demonstrated that *in vivo* insulin action is significantly correlated with the histologically determined proportions of muscle fiber types (26). On the other hand, it has also been reported that testosterone produced dramatic insulin resistance as well as changes in muscle morphology toward a less-red insulin-sensitive type I fiber and a more-white insulin-insensitive type II fiber in rats (27). These findings indicate that an interchange may occur between type I and type II muscle fibers. The results of the present study demonstrated that insulin resistance in FFR is concomitant with changes in muscle fiber composition and that there is a correlation between insulin sensitivity and the composite ratio of type I or type II fibers in the soleus muscle. However, details of the mechanisms by which the composite ratio of type I fiber is reduced in FFR are still unknown. We have confirmed that both a long-acting dihydropyridine Ca channel antagonist (12) and TSJN (21) not only improve insulin sensitivity but also lead to recovery in muscle fiber composite ratios in the FFR. It has also been reported that hyperinsulinemia can lead to a change in muscle fiber composition from type I to type II fibers in the soleus muscle (28). Considering the results of these studies, it is possible that compensatory hyperinsulinemia changes the muscle fiber composition from type I fibers to type II fibers and that a Ca channel antagonist, TSJN, and JTKL improve insulin sensitivity and cancel hyperinsulinemia in FFR, resulting in recovery of muscle fiber composite ratios to the level of those in control rats. However, the mechanisms by which JTKL improves insulin sensitivity remain to be clarified.

TNF- α in Muscle

We previously showed that skeletal muscle TNF- α , not adipose tissue TNF- α , is linked to insulin resistance in FFR (19). There is evidence that fructose feeding for 6 weeks leads to insulin resistance and an increase in muscle TNF- α , and it is known that a negative correlation exists between insulin sensitivity and muscle TNF- α . Some studies have shown that diabetic patients and insulin-resistant obese patients have higher adipose and muscle tissue-derived TNF- α levels and that there is a negative correlation between the level of TNF- α mRNA expression and glucose clearance rate (13, 17). In addition, Hotamisligil *et al.* (13) demonstrated that neutralization of TNF- α with a recombinant soluble TNF- α receptor improved insulin sensitivity in fa/fa rats. TNF- α has been demonstrated to inhibit insulin-responsive glucose uptake through a decrease in glucose transporter 4 gene transcription (29) and a reduction in tyrosine kinase activity of the insulin receptor (30, 31). A negative correlation between insulin sensitivity and skeletal muscle TNF- α level was also found in this study. These results suggest that the level of muscle TNF- α plays a significant role in insulin resistance and regulates glucose transport in skeletal muscle in an autocrine or a paracrine fashion. We previously demonstrated that angiotensin II is one of the factors that might regulate muscle TNF- α (19), although further studies will be needed to elucidate the precise mechanism by which TNF- α is regulated.

Chinese Medicine and Insulin Resistance

There have been few reports on the effects on insulin sensitivity of Chinese medicines used for treatment of diabetes mellitus. Kataoka *et al.* reported that the Chinese medicine QX dramatically improved blood glucose levels in type 2 diabetes mellitus patients who had already been treated with a maximum dose of glibenclamide, and that there were no differences in urinary excretions of C-peptide after treatment. They therefore speculated that QX might improve insulin sensitivity, not insulin secretion (32). We previously reported that the Chinese medicine TSJN improved insulin sensitivity in FFR, partially due to a change in skeletal muscle fiber composition—*i.e.*, a change to more type I and less type II fibers (21). The Chinese medicine JTKL appeared to improve insulin sensitivity in FFR concomitant not only with a decrease in muscle tissue TNF- α but also with a change in skeletal muscle fiber composition.

JTKL contains several crude drugs, PG, *Polygonum multiflorum thunb.*, and *Curcuma zedoaria* Rose, which might have antiatherosclerotic effects. *Asparagus corhinchinensis*, *Pucraria pseudo-hirstua*, and *Rehmannia glutiosa libosch* are believed to have moderate blood glucose-lowering actions, and *Pucraria pseudo-hirstua* is also believed to have a vasodilative effect. It seems that PG, as one of the valid components in JTKL, plays a significant role in the improvement

of insulin resistance in FFR. It is well known that some Chinese medicines also have side effects, but there has been no report of any severe side effects of JTKL. Since crude drugs, including JTKL, PG, *Rheum rhubarb*, *Rehmannia glutiosa libosch*, and *Chinese cinnamon*, have been used in China, Japan, and other countries, JTKL could be used to treat insulin-resistant patients.

In summary, development of insulin resistance and hypertension in FFR is concomitant with an increase in TNF- α and a change to a smaller ratio of type I fibers in skeletal muscles. The Chinese medicine JTKL, which contains PG as one of its valid components, might play a significant role in improvement of insulin resistance. Treatment with JTKL results in a decline in muscle tissue TNF- α and a recovery of muscle fiber composition. It is possible that JTKL lowers muscle tissue TNF- α and improves insulin sensitivity, then cancels compensatory hyperinsulinemia in FFR, resulting in recovery of the muscle fiber composite ratio to the level of that in control rats. However, the precise mechanisms by which JTKL improves insulin sensitivity remain to be clarified. The results of this study suggest that the TNF- α level in skeletal muscles and muscle fiber composition play important roles in the development of insulin resistance in FFR and that treatment with JTKL is beneficial for insulin-resistant hypertensives.

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