

Effect of Tangzhiqing on Glucose and Lipid Metabolism in Genetically Type 2 Diabetes KK-Ay Mice

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The hypoglycemic and hypolipidemic effects of Tangzhiqing (abbreviated TZ) a mixture of Mulberry Leaf, Lotus Leaf, Danshen Root, and Hawthorn Leaf, were investigated in KK-Ay mice, an animal model of type 2 diabetes. TZ reduced blood glucose, total cholesterol, and triglyceride levels of KK-Ay mice at 4 weeks after oral administration. TZ also improved sucrose or maltose tolerance. Maltase activity in small intestine significantly decreased in the TZ-treated KK-Ay mice. These results support the hypothesis that TZ improves glucose metabolism by reducing α -glycosidase activity. Therefore TZ might have beneficial effects on hyperglycemia and hyperlipidemia in type 2 diabetes.

Key words—Tangzhiqing, KK-Ay mice, sucrase, maltase, traditional Chinese medicine

INTRODUCTION

Despite considerable progress in the manage-

ment of diabetes mellitus by synthetic drugs, the search for natural antidiabetic agents is ongoing. The plant kingdom offers a wide field to look for effective oral hypoglycemics. More than 400 species have been reported to display hypoglycemic effects, but only a few of them have been thoroughly investigated.^{1–3)} Many traditional Chinese antidiabetic medicines need further study and development.

Tangzhiqing (abbreviated TZ) is a traditional Chinese medicine that is widely used to treat diabetes mellitus. However, adequate characterization of antidiabetic activity has yet to be done and few studies have been performed on type 2 diabetes models.

In the present study, we examined the effect of TZ in KK-Ay mice, an animal model of genetic type 2 diabetes.

MATERIALS AND METHODS

TZ was obtained from the Department of Traditional Chinese Medicine of Tianjin University, China. TZ is a prescription that consists of Mulberry Leaf 25%, Lotus Leaf 25%, Danshen Root 25%, and Hawthorn Leaf 25%. The decoction extractive was used in the experiment. After filtration, water extracts (100°C, 45 min) were lyophilized and stored at room temperature until use. The yield was 8.8%.

Animals—KK-Ay strain male mice (8 weeks old; CLEA Japan, Osaka, Japan) were used in this study. The mice were housed in an air-conditioned room at 22 ± 2°C with a 12 hr light-12 hr dark cycle (light: 9:00a.m. to 9:00p.m.). The animals were kept in an experimental animal room for 7 days with free access to food (CE-2, CLEA Japan) and water (tap water). The mice were divided into four groups: control, high dosage (0.7 g/kg), middle dosage (0.35 g/kg), and low dosage (0.175 g/kg) of TZ. The animals were treated orally once daily for 4 weeks. Blood samples were drawn from a cavernous sinus capillary to determine blood glucose levels under non-anesthesia and fasting. TZ was dissolved in distilled water. Distilled water alone was administered the control mice. The studies were started at 9:00–10:00a.m. and blood samples after repeated administration of TZ were then taken. TZ was administered orally on a compulsory basis (repeated administration, once daily). Animals were treated in accordance with the Guideline for the Care and Use of Laboratory Animals (the Prime

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Minister's Office No. 6, 1980).

Oral Sugar Tolerance Test — A sugar tolerance test was performed at the end of repeated administration. After overnight (18 hr) fasting, either sucrose or maltose (1 g/kg) solution was administered orally and blood samples were obtained before and 30, 60, and 120 min after administration and glucose content was determined.

Sucrase, Maltase and Lactase Activity — Glycosidase activity was assayed at the end of repeated administration. Mouse small intestine was excised, and mucous membrane was removed on a slide and homogenized individually with Potter-Elvehjem homogenizer. Sucrase, maltase, and lactase activity was assayed by the Dahlqvist method⁴⁾ with some modifications. Sucrose, maltose, and lactose (50 mM) was incubated with diluted enzyme at 37°C for 30 min. The reaction was terminated by placing in a boiling water bath for 3 min and glucose content was determined.

Determination of Plasma Total Cholesterol, Triglyceride, Glucose, and Insulin — Plasma total cholesterol, triglyceride, glutamic oxaloacetic transaminase (GOT) levels, and blood glucose levels in mice were determined using commercial reagents⁵⁾ (Glucose C-II test Wako; Triglyceride E test Wako; Cholesterol E test Wako; Fuji Dri Chem SD slide GOT). The plasma insulin was measured by double-antibody method.⁶⁾

Statistical Analysis — All data are expressed as mean \pm S.E. Student's *t*-test and analysis of variance (ANOVA) followed by SPSS was used for statistical analysis. The values were considered significant when the *p*-value was < 0.05 .

RESULTS

Effect of TZ on Blood Glucose, Insulin, and Body Weight in KK-Ay Mice (Repeated Administrations)

The effect of repeated administration of TZ on

blood glucose is shown in Table 1. TZ treated KK-Ay mice of 0.35 g/kg and 0.7 g/kg dosage groups showed a reduction in blood glucose levels 4 weeks after administration. The plasma insulin level in TZ-treated KK-Ay mice 4 weeks after administration was reduced. The effect of repeated administration of TZ on body weight is shown in Fig. 1. After 4 weeks of repeated administration of TZ, body weight of KK-Ay mice did not change (TZ 0.175 g/kg vs. Control, $p = 0.12$; TZ 0.35 g/kg vs. Control, $p = 0.22$; TZ 0.7 g/kg vs. Control, $p = 0.15$).

Effect of TZ on Oral Sugar Tolerance Test in KK-Ay Mice

The effect of TZ on oral sucrose tolerance test is shown in Fig. 2. TZ at high dosage (0.7 g/kg), middle dosage (0.35 g/kg), and low dosage (0.175 g/kg) improved hyperglycemia after sucrose loading (30, 60, 120 min, $p < 0.01$).

The effect of TZ on oral maltose tolerance test is shown in Fig. 3. TZ at high dosage (0.7 g/kg), middle dosage (0.35 g/kg), and low dosage (0.175 g/kg) improved hyperglycemia after maltose loading (0.175, 0.35 mg/kg, 30, 60 min,

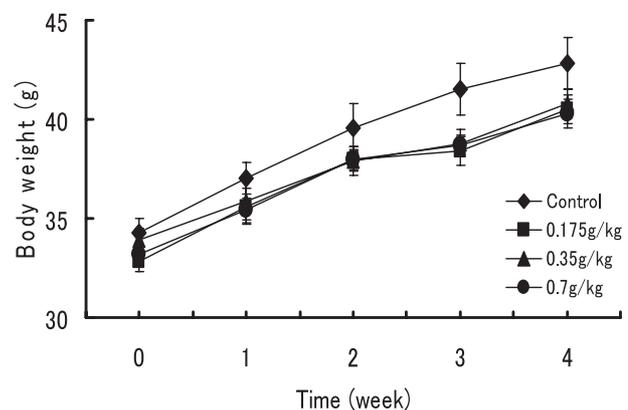


Fig. 1. Effect of TZ on Body Weight

TZ high dosage (0.7 g/kg), middle dosage (0.35 g/kg), and low dosage (0.175 g/kg) TZ was administered orally to KK-Ay mice. Each value represents the mean \pm S.E. of 6–8 mice.

Table 1. Effect of Tangzhiqing (TZ) on Blood Glucose, Lipid, and α -glucosidase Activity in KK-Ay Mice

Group	<i>n</i>	Blood glucose level (mg/dl)	Insulin (μ U/ml)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Sucrase (%)	Maltase (%)	Lactase (%)
Control	6	222 \pm 6	87 \pm 20	162 \pm 7	307 \pm 22	100 \pm 21	100 \pm 23	100 \pm 22
0.175 g/kg	8	212 \pm 4	40 \pm 8*	137 \pm 6*	179 \pm 23**	54 \pm 10	49 \pm 11*	64 \pm 11
0.35 g/kg	8	184 \pm 4*	43 \pm 10*	138 \pm 6*	224 \pm 14**	55 \pm 24	26 \pm 13**	58 \pm 23
0.7 g/kg	8	184 \pm 2*	23 \pm 11**	142 \pm 6*	165 \pm 15**	53 \pm 10	12 \pm 8**	63 \pm 12

Each value represents the mean \pm S.E. Significantly different from controls, * $p < 0.05$, ** $p < 0.01$.

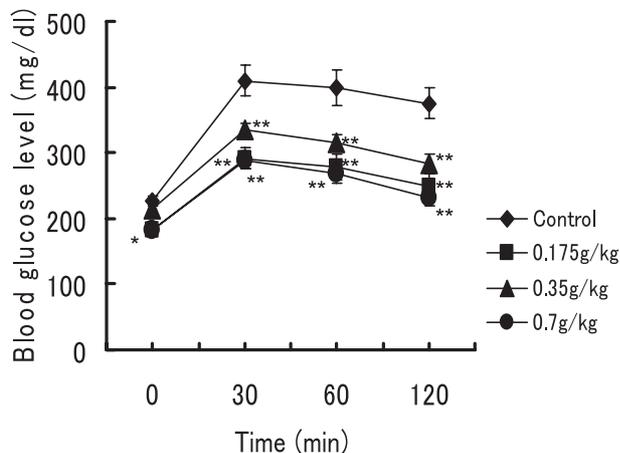


Fig. 2. Effect of TZ on Oral Sucrose Tolerance after 4 Weeks of Repeated Administration in KK-Ay Mice

After overnight (18 hr) fasting, sucrose (1 g/kg body weight) solution was administered orally. Blood samples were collected before and 30, 60, and 120 min after administration. Each value represents the mean \pm S.E. of 6–8 mice. Significantly different from corresponding controls, * $p < 0.05$; ** $p < 0.01$.

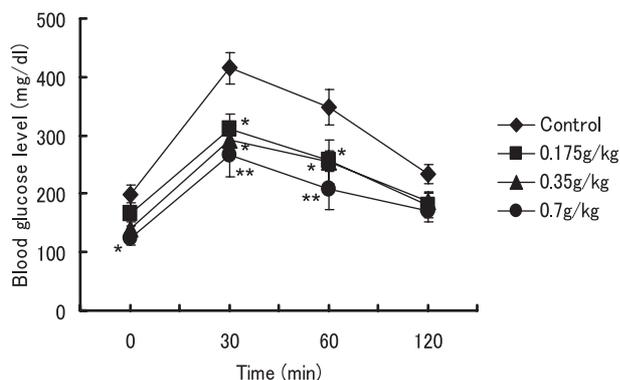


Fig. 3. Effect of TZ on Oral Maltose Tolerance after 4 Weeks of Repeated Administration in KK-Ay Mice

After overnight (18 hr) fasting, maltose (1 g/kg body weight) solution was administered orally. Blood samples were collected before and 30, 60, and 120 min after administration. Each value represents the mean \pm S.E. of 6–8 mice. Significantly different from corresponding controls, * $p < 0.05$; ** $p < 0.01$.

$p < 0.05$; 0.7 mg/kg, 30, 60 min, $p < 0.01$).

Effect of TZ on Sucrase, Maltase, and Lactase Activity in Small Intestine of KK-Ay Mice

The effects of TZ on sucrase, maltase, and lactase activity in small intestine are shown in Table 1. TZ significantly reduced maltase activity in small intestine of KK-Ay mice. However, TZ did not reduce lactase activity in small intestine of KK-Ay mice.

Effects of TZ on Plasma Total Cholesterol and Triglyceride of KK-Ay Mice

TZ in all three dosage groups decreased plasma cholesterol level at 4 weeks after oral administration when compared with controls ($p < 0.01$; Table 1). TZ 0.175–0.7 g/kg dosage groups decreased plasma triglyceride at 4 weeks when compared with controls ($p < 0.01$).

DISCUSSION

This study clearly showed that water extract of TZ produces a consistent hypoglycemic effect. We studied the effect of TZ in KK-Ay mice at high dosage (0.7 g/kg), middle dosage (0.35 g/kg), and low dosage (0.175 g/kg), and examined the therapeutic effects of TZ on hyperglycemia in KK-Ay mice, an animal model of type 2 diabetes mellitus. These mice, which are known for genetically induced diabetes and which include ob/ob mice⁷⁾ and KK mice,⁸⁾ had hyperinsulinemia as a result of insulin resistance.⁹⁾ Their treatment with TZ resulted in hypoglycemia and reduced plasma insulin. These results indicate that TZ may be useful for type 2 diabetes.

Further, we found that TZ reduced maltase activity (α -glucosidase) in the small intestine. However, TZ did not change lactase activity (β -galactosidase). From this finding, it seems likely that TZ inhibits sugar absorption by decreasing α -glucosidase activity. In addition, TZ improved hyperinsulinemia in KK-Ay mice. It is known that α -glucosidase inhibitors improve insulin resistance.^{10–12)} These findings suggest that the antidiabetic activity of TZ is derived, at least in part, from an improvement of insulin resistance, due to reduction of α -glucosidase activity.

TZ did not change the body weight and blood GOT level of KK-Ay mice by repeated administration [GOT levels: Control 48 ± 9 , TZ 0.175 g/kg 42 ± 8 , TZ 0.35 g/kg 38 ± 15 , TZ 0.7 g/kg 43 ± 8 (U/I)]. This finding suggests that TZ is a supplement with low toxicity. Moreover, this study clearly showed that TZ reduced plasma total cholesterol and plasma triglyceride in KK-Ay mice. Diabetics also often have elevated blood cholesterol and triglyceride.¹³⁾ From these findings, it seems likely that TZ exerts hypolipidemic activity by decreasing insulin resistance. Therefore TZ might exert beneficial effects on hyperglycemia and hyperlipemia in type 2 diabetic subjects.

Because traditional Chinese medicines contain many compounds, further studies are needed to clarify the details. Further study would show how TZ could become a useful drug in the treatment of diabetes through this unique therapeutic mechanism. The above experimental results supports the traditional Chinese medicine view of TZ as a treatment for type 2 diabetes.

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