Hypoglycemic Activity of To-Kai-San (Chinese Medicines) in Normal and KK-Ay Mice

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Summary The hypoglycemic effect of To-Kai-San (TS) was studied in normal mice, streptozotocin-induced diabetic mice, one of the animal models of insulin-dependent diabetes mellitus (IDDM) with hypoinsulinemia, and KK-Ay mice, one of the animal models of non-insulin-dependent diabetes mellitus (NIDDM) with hyperinsulinemia. TS (1,500 mg/kg) reduced the blood glucose of the KK-Ay mice from 441 ± 36 to 285 ± 52 mg/100 ml 7 h after a single oral administration ($p < 0.001$). However, no changes in blood glucose were observed in the normal and STZ-induced diabetic mice. TS also decreased the blood glucose 20 days after repeated administration to normal and KK-Ay mice. In addition, TS-treated KK-Ay mice showed decreased plasma insulin levels after both single and repeated administration. From these findings, it is believed that TS may require the presence of insulin to display its activity.

Key Words hypoglycemic activity, To-Kai-San, normal mice, KK-Ay mice

The traditional use of medicinal plants for the treatment of diabetes mellitus greatly declined in developed countries after the introduction of insulin in 1921. In the last few years, serious attempts have been made to develop new easy-to-use, non-parenteral forms of insulin, but the attempts have yielded poor results to date. On the other hand, there is a growing need for new oral antidiabetic drugs such as CS-045 (1) to serve as an alternative therapy in non-insulin-dependent diabetes mellitus (NIDDM). The plant kingdom is 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 investigated (2–4). Under the present circumstances, many reports about diabetes agents were investigated using streptozotocin, an insulin-dependent diabetes mellitus (IDDM); however, very few reports about NIDDM have been presented.

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Table 1. Composition of To-Kai-San.

	To-Kai-San (%)
Bakumontou (the tuber of <i>Ophiopogon japonicus</i> Ker-Gawler)	12.0
Ninjin (the radix of <i>Panax ginseng</i> C. A. Meyer)	8.0
Kakkon (the radix of <i>Pueraria lobata</i> Ohwi)	12.0
Jiou (the radix of <i>Rhmannia glutinosa</i> Liboschitz ver)	12.0
Chimo (the rhizoma of <i>Anemarrhena asphodeloides</i> Bunge)	10.0
Karokon (the radix of <i>Trichosanthes kirilowii</i> Maximowicz)	12.0
Bukuryou (<i>Poria cocos</i>)	12.0
Gomishi (the fruit of <i>Schisandra chinensis</i> Baillon)	6.0
Kanzou (the radix of <i>Glycyrrhiza uralensis</i> Fisher)	6.0
Tarakon (the radix of <i>Aralia elata</i> Seemann)	10.0
	100.0

Many years ago, diabetes was not diagnosed because there was no measurement method for insulin. However, many medicines have been used for conditions like diabetes, polyurea, polydipsia and dehydration. From this point of view, some of them applied to diabetic agents.

To-Kai-San (TS) has been used as a medicine for diabetes. The constituents of TS have been indicated to include 10 raw medicines (Table 1) (5). TS has been clinically taken at the rate of one dose (2 g) three times a day during each meal. However, there is no experimental evidence of the hypoglycemic effect of this medicine. Furthermore, it is not known whether TS will be effective for IDDM or NIDDM. The purpose of this study was to examine the hypoglycemic effect using two types of diabetic mice.

MATERIALS AND METHODS

Materials. To-Kai-San (crude powder extract, TS) was obtained from Mayado Co., Hyogo, Japan. The TS properties include: (1) heavy metal by the J.P. test; (2) the water extract is more than 55%; and (3) the ash content is less than 7.0%. The Glycyrrhizae radix of TS involved 3–7% glycyrrhizin. The TS was dissolved in distilled water.

Animals. Adult male ddY mice (SLC, Shizuoka, Japan), 5-weeks-old and weighing 22–25 g, were used. The mice were housed in an air-conditioned room at $22 \pm 2^\circ\text{C}$ with a 12 h light/dark cycle (light: 9:00 am to 9:00 pm). The animals were kept in the experimental animal room for seven days with free access to food (Table 2) and water (tap water). For the determination of blood glucose and insulin levels, blood samples were withdrawn from the cavernous sinus with a capillary under non-anesthesia and nonfasting. All of the experiments were conducted under these conditions.

Streptozotocin-induced diabetic mice were produced by injecting the mice

Table 2. Composition of the diets (%).

Ingredients	
Casein	25.0
DL-Methionine	0.3
Corn starch	44.7
Sucrose	10.0
Corn oil	7.0
Cellulose	5.0
Vitamin mix	1.0
Mineral mix	7.0
Total	100.0

intravenously with 150 mg/kg body weight streptozotocin (STZ, Sigma) (150 mg STZ dissolved in 10 ml 0.1 M citrate buffer, pH 4.5). After the administration of STZ, the animals were kept with free access to food and water. Eight days after the injection of STZ, the blood glucose levels of all the mice were determined.

Male KK-Ay mice, 12-weeks-old and weighing 39–43 g, were used. STZ and KK-Ay mice with blood glucose levels above 300 mg/100 ml two days before the experiment were considered to be diabetic and were used in this study. Five to six animals were used for each group. The studies were started at 10:00–11:00 am, and the blood samples after repeated administration of TS were taken at 10:00–11:00 am. The oral administrations of TS were given on a compulsory basis. TS concentrations (150, 500 and 1,500 mg) were dissolved in 20 ml distilled water. All the experimental conditions for TS were non-fasting, and free access to food and water.

Determination of blood glucose, cholesterol and triglyceride. Blood glucose, cholesterol, triglyceride and insulin levels were determined using commercial reagents ("Glucose C-Test Wako," "Cholesterol E-Test Wako," "Triglyceride G-Test Wako" and "Insulin-EIA Test," Wako Pure Chemical Industries, Ltd.). All the data were expressed as $M \pm SEM$ and Student's *t*-test was used for the statistical analysis. The values were considered to be significantly different when the *p* value was less than 0.05.

RESULTS

Effect of a single administration of TS on normal mice, streptozotocin-induced diabetic mice (IDDM) and KK-Ay mice (NIDDM)

The mean blood glucose levels of the KK-Ay mice at various time intervals after the oral administration of TS are shown in Fig. 1. TS at 1,500 mg/kg lowered the blood glucose level from 441 ± 36 to 285 ± 52 mg/100 ml ($p < 0.05$) 7 h after administration. TS at 500 mg/kg decreased the blood glucose level from 500 ± 48 to 263 ± 39 mg/100 ml ($p < 0.05$) 7 h after administration. The plasma insulin levels in

Fig. 1. Effect of TS on blood glucose in KK-Ay mice. Each point indicates the $M \pm SE$ of 4-6 mice. Significantly different from prevalue, a) $p < 0.05$, b) $p < 0.01$. ○, control; △, TS 150 mg/kg; □, TS 500 mg/kg; ●, TS 1,500 mg/kg.

Fig. 2. Effect of TS on insulin concentration in KK-Ay mice. Each value represents the $M \pm SE$ of five mice. Significantly different from TS 0 mg/kg, a) $p < 0.05$. ○, TS 0 mg/kg; ●, TS 1,500 mg/kg.

the TS-treated (1,500 mg/kg) mice decreased at 7 h (Fig. 2), indicating that TS improved hyperinsulinemia in KK-Ay mice.

On the other hand, there was no significant difference in the levels between the control and TS-treated groups in normal mice (control (mg/100 ml): 190 ± 11 at 0 h, 202 ± 1 at 2 h, 188 ± 12 at 4 h, 184 ± 8 at 7 h, 176 ± 11 at 10 h; TS: 193 ± 8 at 0 h, 180 ± 5 at 2 h, 174 ± 7 at 4 h, 167 ± 6 at 7 h, 166 ± 9 at 10 h). In addition, no changes in blood glucose were observed in the TS-treated (1,500 mg/kg) STZ-induced diabetic mice (control (mg/100 ml): 589 ± 22 at 0 h, 531 ± 44 at 2 h, 495 ± 27 at 4 h, 527 ± 53 at 7 h, 512 ± 40 at 10 h; TS (mg/100 ml): 632 ± 15 at 0 h, 533 ± 14 at 2 h, 543 ± 13 at 4 h, 538 ± 27 at 7 h, 597 ± 49 at 10 h).

Effect of repeated administration of TS on normal, STZ and KK-Ay mice

The effect of the repeated administration of TS on the blood glucose level in normal mice is shown in Fig. 3. TS-treated (1,500 mg/kg) mice showed a significant decrease in blood glucose level after 20 days when compared with control mice. However, there were no significant differences in the levels of total cholesterol and triglyceride between the control and TS-treated groups (Total cholesterol: 156 ± 10 and 156 ± 7 mg/100 ml for control and TS, respectively; triglyceride: 151 ± 7 and 162 ± 12 mg/100 ml for control and TS, respectively). The mean blood glucose levels in KK-Ay mice after 20 days are shown in Fig. 4. TS-treated (1,500 mg/kg) KK-Ay mice had a decreased blood glucose level after 20 days (538 ± 8 and 348 ± 43 mg/100 ml for control and TS, respectively, $p < 0.01$) (Fig. 4) as well as decreased insulin level (391 ± 11 and 319 ± 21 μ U/ml for control and TS, respectively,

Fig. 3

Fig. 4

Fig. 3. Effect of repeated administration of TS on blood glucose in normal mice. Each point indicates the $M \pm SE$ of 10–12 mice. Significantly different from the prevalence, c) $p < 0.001$. $\circ$, TS 0 mg/kg; $\bullet$, TS 1,500 mg/kg.

Fig. 4. Effect of repeated administration of TS on blood glucose in KK-Ay mice. Each point indicates the $M \pm SE$ of five mice. Significantly different from TS 0 mg/kg, b) $p < 0.01$. $\circ$, TS 0 mg/kg; $\bullet$, TS 1,500 mg/kg.

Fig. 5. Effect of repeated administration of TS on insulin concentration in KK-Ay mice. Each value represents the $M \pm SE$ of five mice. Significantly different from TS 0 mg/kg, b) $p < 0.01$.

$p < 0.01$) (Fig. 5). However, TS-treated (1,500 mg/kg) STZ mice did not show a decrease in blood glucose (804 ± 9 and 857 ± 8 mg/100 ml for control and TS, respectively).

DISCUSSION

This study clearly shows that TS produced consistent hypoglycemic effects in normal and KK-Ay mice, one of the animal models of non-insulin-dependent diabetes mellitus (NIDDM) with hyperinsulinemia. KK-Ay mice, which are known for genetically induced diabetes, including ob/ob mice (6) and KK mice (7), were hyperinsulinemia as a result of insulin resistance (8). However, TS (1,500 mg/kg) had no effect on the blood glucose levels of STZ-induced diabetic mice, one of the animal models of insulin-dependent diabetes mellitus (IDDM) with hypo-insulinemia (9). TS-treated KK-Ay mice showed decreased blood glucose level and improved hyperinsulinemia, indicating that TS may relax the insulin resistance. The effect of TS concentrations of 500 and 1,500 mg/kg was not different in KK-Ay mice. From this finding, a TS concentration of 500 mg/kg is the maximum dose for the hypoglycemic effect. In addition, the repeated administration of TS decreased the blood glucose levels in normal and KK-Ay mice for 20 days. However, the repeated administration of TS did not decrease the blood glucose levels in streptozotocin-induced diabetic mice. From these findings, it is believed that TS may require the presence of insulin to display its activity. It is possible to elevate insulin sensitivity in peripheral tissues, because TS decreases the blood glucose level in normal mice. In terms of a hypoglycemic effect, the blood glucose level after a single administration was lower than that in KK-Ay mice after repeated administrations, suggesting that TS enhanced the metabolic enzyme. The dose of 1,500 mg/kg is ten times that for humans. Many traditional medicines decrease the blood glucose level in such a high dose as this; for example, *Centaurea corubionensis*

(2.5–10 g/kg) (10) and *Momordica charantia* (4 g/kg) (11).

In regards to toxicity, the toxicity of TS seems to be very low ($LD_{50} \gg 1,500$ mg/kg) (data not shown). Moreover, TS-treated (1,500 mg/kg) mice did not show any obvious stimulus action, suggesting that TS is a medicine with lower toxicity.

In conclusion, the hypoglycemic effect of TS differs according to the type of diabetes.

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