The Effects of a Traditional Medicine, Fang-ji-huang-qi-tang (Boi-ogi-to), on Urinary Sugar and Sugar Alcohols in Streptozotocin-induced Diabetic Mice

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Boi-ogi-to (Fang-ji-huang-qi-tang, FJHQ) is a Kampo Medicine which has been clinically used in the treatment of arthritis and edema, and includes Astragali Radix, Atractylodes Lanceae Rhizoma, Glycyrrhizae Radix, Zingiberis Rhizoma and Zizyphi Fructus combined with Sinomeni Caulis et Rhizoma (SCR) in Japan, but with Stephania Radix (SR) in China as a substitute for SCR. We have previously reported that FJHQ containing SR [FJHQ(SR)] decreases high blood glucose levels and the elevated blood immunoreactive insulin level in streptozotocin-induced diabetic mice to a greater extent than FJHQ having SCR [FJHQ(SCR)]. In the present study, we show that FJHQ(SR) strongly decreases urinary glucose, sorbitol, fructose, *myo*-inositol and 1,5-anhydro-D-glucitol in STZ-diabetic mice; the effect was markedly stronger than that of FJHQ(SCR), indicating that FJHQ(SR) suppresses polyol pathway activity. The present data support the hypothesis that FJHQ(SR) may inhibit the development of diabetic complications.

Key words —— Fang-ji-huang-qi-tang, diabetes, sugar, sugar alcohol, antihyperglycemic effect, polyol pathway

INTRODUCTION

Boi-ogi-to (Fang-ji-huang-qi-tang, FJHQ) is a traditional prescription which consists of six crude drugs: Sinomeni Caulis et Rhizoma (SCR), Astragali Radix, Atractylodis Lanceae Rhizoma, Glycyrrhizae Radix, Zingiberis Rhizoma and Zizyphi Fructus in Japan. Although Stephania Radix (SR) is used as a substitute for SCR in FJHQ in China, FJHQ has been clinically used in the treatment of arthritis and edema in both Japan and China. Since FJHQ(SCR) has been reported to inhibit the elevation of serum cholesterol and decrease the ratio of visceral fat to somatic fat in diabetic patients with obesity,[1] it is possible that FJHQ(SR) has a similar or more beneficial effect in improving diabetes.

Streptozotocin (STZ)-induced diabetes mellitus has been widely accepted as a model of insulin-dependent diabetes mellitus.[2] However, STZ-diabetic rodents still have low insulin levels and survive for some months. Recently, several investigators have reported that a single treatment of low doses of STZ induces a non-insulin-dependent diabetes mellitus (NIDDM)-like hyperglycemic state in both neonatal and adult rodents.[3–5] This diabetic model characteristically shows mild hyperglycemia and impaired pancreatic function, accompanied by insulin resistance. In addition, the oral administration of antidiabetic drugs such as tolbutamide and glibenclamide improves hyperglycemia and increases the blood insulin level in STZ-diabetic rodents.[6–8] These characteristics of STZ-diabetic mice are very similar to those of NIDDM.

The chronic complications of diabetes, including retinopathy, neuropathy and nephropathy,[9] are the major causes of morbidity and mortality associated with the disease. It has been shown that diabetic complications may result from damage of cellular function caused by abnormal polyol metabolism.[10–13] In other words, the complications in poorly controlled diabetes would be ultimately caused by abnormal polyol metabolism[14] as well as glycation of proteins,[15,16] because the modification of nucleic acids and proteins by polyol can be due to activation of the hyperglycemia-induced polyol pathway.[17]

In our previous studies, it was found that high blood glucose is reduced, whereas blood immunoreactive insulin is elevated in STZ-diabetic mice after

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the administration of FJHQ(SR).[18,19] However, it is unclear whether FJHQ(SR) is effective on the polyol pathway activity which is elevated in diabetes. To address this question, we analyzed the urinary levels of glucose, fructose, *myo*-inositol and 1,5-anhydro-D-glucitol in STZ-diabetic mice treated with or without FJHQ(SR), by gas chromatography mass spectrometry with some modifications.[20,21] In addition, the effects of FJHQ(SR) was compared to those of FJHQ(SCR), since it is possible that these two FJHQs have different effects on the polyol pathway in diabetes.

MATERIALS AND METHODS

Preparation of Streptozotocin-Diabetic Mice —— Male mice (ddY strain; 5 weeks of age; body weight, 22–25 g; Kiwa Laboratory Animal Science Co., Ltd. Wakayama, Japan) were injected with a single dose (150 mg/kg, i.v.) of streptozotocin (STZ: Sigma, St. Louis, MO, U.S.A.) in saline into the tail vein. STZ-induced diabetic mice (8–9 weeks of age; body weight, 22–46 g; blood glucose levels, 300–700 mg/dl) were used for experiments 3–4 weeks after the injection. Age-matched normal male mice (ddY strain; 8–9 weeks of age; body weight, 29–42 g; blood glucose level, 88–179 mg/dl) were used as the control. These mice were housed at 25–26°C with lights on from 7 a.m. to 7 p.m. and given a normal laboratory diet (PMI Lab Diet, Japan SLC, Shizuoka, Japan) and water *ad libitum*.

Preparation and Administration of Crude Drugs —— FJHQ(SR) was composed of the following six herbs: SR (5 parts), Astragali Radix (5 parts), Atractylodis Lanceae Rhizoma (3 parts), Glycyrrhizae Radix (1.5 parts), Zingiberis Rhizoma (1 parts) and Zizyphi Fructus (3 parts). SCR (5 parts) was used as a substitute for SR in FJHQ(SCR). These crude drugs were collected from the following places: SR from Anhui shen, China, during autumn; SCR from Tokushima and Kagawa prefectures, during November to March; Astragali Radix from Neimeng gu, China, during autumn; Atractylodis Lanceae Rhizoma from Hubei shen, China, during autumn; Glycyrrhizae Radix from Xinbei bu, China, during autumn; Zingiberis Rhizoma from Guizhou shen, China, during autumn; Zizyphi Fructus from Henan shen, China, during autumn. The composition of either FJHQ(SR) or FJHQ(SCR) was extracted in 6 volumes of distilled water at 96–98°C for 40 min with an automatic extractor (Torobi, Tochimoto, Osaka, Japan). The water extracts were filtered through a mesh (No. 42, Sanpo, Tokyo, Japan), lyophilized with a freeze-drier (DF-03G, ULVAC, Tokyo, Japan), and stored at 4°C. These extracts were suspended homogeneously in saline containing 1% Avicel (Asahi Chemical Industry, Tokyo, Japan),[18] and were intraperitoneally (i.p.) (0.1 ml/10 g body weight) injected into 12 hr-fasted mice.

Measurement of Serum Glucose Levels —— Blood samples were collected from the orbital vein plexus of mice before and 12 hr after i.p. administration of the extract of either FHJQ(SR) or FHJQ(SCR). Blood samples were collected and then centrifuged at 3000 rpm at 4°C for 20 min. The glucose level of the serum was measured by the glucose oxidase method using a glucose *b*-test (Wako, Osaka, Japan).

Measurement of Urinary Sugar and Sugar Alcohols —— Urine was collected during 12 hr after drug administration. Urine samples were prepared using a urease treatment method as follows:[20,21] One hundred μl of urine was incubated with 30 units of urease at 37°C for 30 min. After adding an internal standard (20 μl of *n*-heptadecanoic acid), the sample was mixed with 900 μl of ethanol and centrifuged to deproteinize it; the supernatant was collected and evaporated. The residue was Completely dried under a nitrogen stream for 5 min and re-extracted with 100 μl of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide and 10 μl of trimethylchlorosilane at 80°C for 30 min. The urinary sugars and sugar alcohols (glucose, sorbitol, fructose, *myo*-inositol and 1,5-anhydro-D-glucitol) were measured by modified gas chromatography mass spectrometry.

Statistical Analysis —— Data are expressed as means ± S.E.M. and statistically analyzed by one-way ANOVA followed by the multiple range tests of Scheff'e, or unpaired *t*-test. A *p*-value of less than 0.05 was considered to indicate a statistically significant difference.

RESULTS

Figure 1 shows the serum glucose level of STZ-diabetic mice treated with or without FJHQ (SR) or FHJQ(SCR). In the control group, the serum glucose remained almost constant at a low level of about 110 mg/dl. The administration of STZ resulted in a marked increase in the glucose level , to approximately 480 mg/dl; FJHQ(SR) (160 mg/kg) signifi-

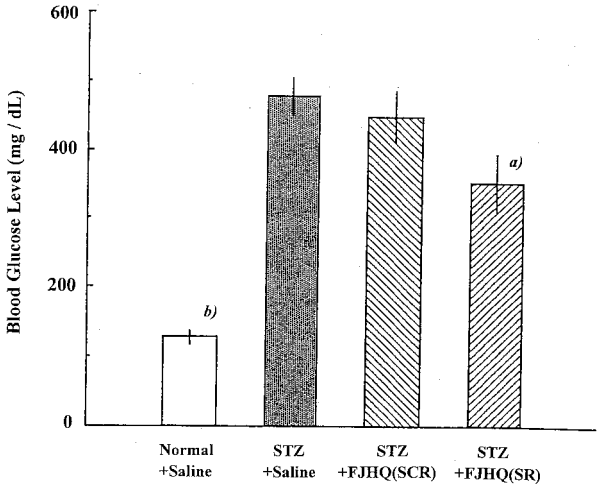

Fig. 1. Blood Glucose Levels of Normal, STZ-Diabetic Mice Treated with or without FJHQ(SR) or FJHQ(SCR)

The blood glucose was measured 12 hr after i.p. administration of FJHQ(SR) or FJHQ(SCR). Significantly different from the STZ-diabetic mice, *a*) $p < 0.05$, *b*) $p < 0.01$.

cantly decreased the glucose level in STZ-diabetic mice. However, FJHQ(SCR) (160 mg/kg) failed to show such a beneficial effect.

Figure 2 shows representative profiles of total ion current chromatograms of urinary sugar and sugar alcohols from control and STZ-diabetic mice treated with or without FJHQ(SR) or FJHQ(SCR). The results from five experiments are summarized in Table 1. It was observed that urinary glucose, sorbitol, fructose, *myo*-inositol and 1,5-anhydro-D-glucitol were markedly increased in the STZ-diabetic mice. When STZ-diabetic mice were treated with FJHQ(SR), these urinary glucose and sugar alcohols were dramatically decreased. On the other hand, FJHQ(SCR) significantly decreased urinary *myo*-inositol , but failed to decrease glucose and other sugar alcohols.

DISCUSSION

We have previously reported that FJHQ(SR) significantly reduced high blood glucose levels and elevated the blood immunoreactive insulin level of STZ-diabetic mice on intraperitoneal and oral administration.[20,21] In the present study, it was found that FJHQ(SR) markedly decreases urinary glucose, sorbitol, fructose, *myo*-inositol and 1,5-anhydro-D-glucitol in STZ-diabetic mice. This indicates that FJHQ(SR) is effective in suppressing an excessively activated polyol pathway in diabetes, as well as hyperglycemia and reduced insulin deficiency. On the other hand, FJHQ(SCR) significantly decreased urinary *myo*-inositol, but failed to decrease urinary glucose and other sugar alcohols, suggesting that the polyol pathway activity can be strongly suppressed

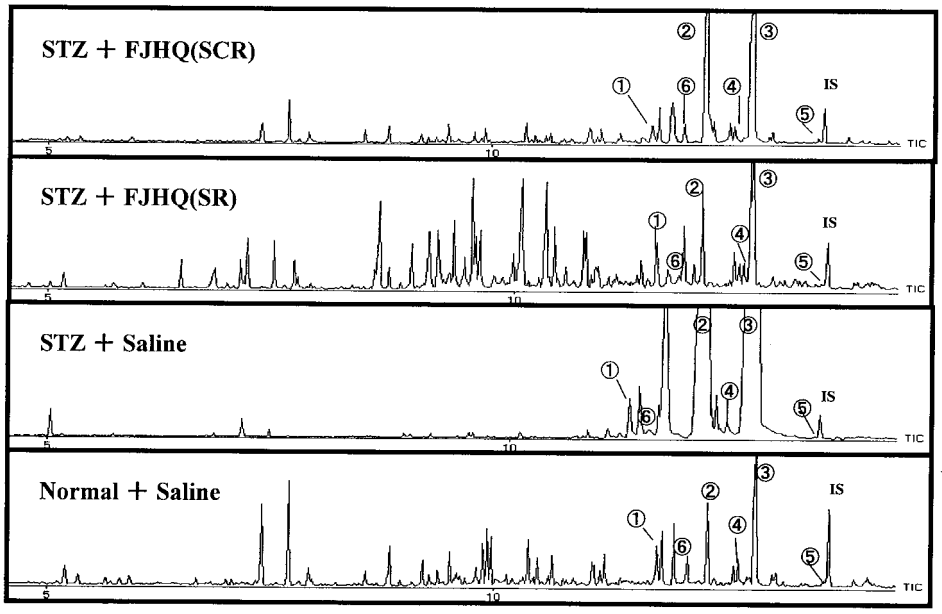

Fig. 2. Total Ion Current Chromatograms of Urinary Sugar and Sugar Alcohols from Normal, STZ-Diabetic Mice Treated with or without FJHQ(SR) or FJHQ(SCR)

1, Fructose; 2, α-Glucose; 3, β-Glucose; 4, Sorbitol; 5, *Myo*-inositol; 6, 1,5-Anhydro-D-glucitol. IS denotes *n*-heptadecanoic acid as an internal standard.

Table 1. Urinary Glucose, Sorbitol, Fructose, *Myo*-Inositol, and 1,5-Anhydro-D-glucitol in the Control and STZ-diabetic Mice Treated with or without FJHQ(SR) or FJHQ(SCR)

	Glucose	Sorbitol	Fructose	*Myo*-inositol	1,5-anhydro-D-glucitol
Control	354 ± 134[a)]	159 ± 49[a)]	48 ± 59[a)]	10 ± 4[a)]	6 ± 3[a)]
STZ	149273 ± 36888	2249 ± 607	4770 ± 1409	254 ± 76	416 ± 113
STZ + FJHQ(SCR)	65975 ± 57472	2179 ± 1921	1931 ± 1739	45 ± 37[a)]	237 ± 211
STZ + FJHQ(SR)	890 ± 534[a)]	202 ± 53[a)]	33 ± 19[a)]	9 ± 2[a)]	7 ± 2[a)]

Values are means ± S.E.M. of five mice. *a*) Significantly different from the STZ-diabetic mice, $p < 0.05$.

by FJHQ(SR), but not by FJHQ(SCR), and the difference is due to the different role of SR and SCR in FJHQ. It is possible that SR but not SCR contains some substance(s) which can suppress the polyol pathway activity in diabetes. On the other hand, it is also possible that the interaction of SR with other crude drugs in FJHQ(SR) is important for the beneficial effects on the pathway. However, these remain to be elucidated.

The polyol pathway is an important biochemical mechanism by which hyperglycemia can impair cell function and structure in diabetic complications,[22–25)] although there are other contributory mechanisms, including the glycosylation of serum proteins, which alters their structure and metabolism, microvascular abnormalities which reduce the availability of oxygen, and changes in platelet function and growth factor activities.[26–29)] In our previous study, it was observed that the FJHQ(SR)-induced reduction of high blood glucose level in STZ-diabetic mice[18)] depends on the elevation of the insulin level in blood.[19)] Although the mechanism by which FJHQ(SR) decreases the polyol pathway activity in STZ-diabetic mice is unclear, an assumption can be made that the FJHQ(SR)-induced improvement of hyperglycemia, which is due to the elevation of blood insulin level, may be responsible for the suppression of the polyol pathway activity.

In conclusion, it was shown that FJHQ(SR) but not FJHQ(SCR) improves glucose and polyol pathway activity in STZ-diabetic mice. The present data support the hypothesis that FJHQ(SR) is a useful medicine for preventing the development of diabetic complications through the suppression of polyol pathway activity.

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