Combined effects of Stephania Radix and Astragali Radix in antihyperglycemic action of Boi-ogi-to (Fang-ji-huang-qi-tang) in streptozotocin-induced diabetic mice

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Abstract

Kampo medicine, Boi-ogi-to (Fang-ji-huang-qi-tang: FJHQ) is composed of different crude drugs between Japan and China to be used clinically in the treatment of arthritis and edema. FJHQ consists of Sinomeni Caulis et Rhizoma (SCR), Astragali Radix (AR), Atractylodes Lanceae Rhizoma, Glycyrrhizae Radix, Zingiberis Rhizoma and Zizyphi Fructus in Japan. Stephania Radix (SR) is a component of FJHQ in China as a substitute for SCR. We have previously reported that FJHQ composed with SR [FJHQ(SR)] decreases high blood glucose levels in streptozotocin (STZ)-induced diabetic mice to a greater extent than FJHQ composed with SCR [FJHQ(SCR)] when they were intraperitoneally administered (Jpn. J. Oriental Med. 49, 607, 1999). The present study investigated roles of combined crude drugs in antihyperglycemic action of FJHQ(SR) in STZ-diabetic mice. FJHQ(SR) by oral administration also showed a significant drop in high blood glucose level and elevated blood immunoreactive insulin level of STZ-diabetic mice. The time course of the maximally elevating action of FJHQ(SR) on blood insulin level occurred earlier than that of antihyperglycemic action. FJHQ without SR and FJHQ without AR did not significantly affect the high blood glucose level, but, FJHQ without the other 4 individual crude drugs have similar actions as that of FJHQ(SR). SR directly decreased high blood glucose level and elevated blood insulin level in the diabetic mice. AR did not have direct effects but potentiated the action of SR on both blood glucose and insulin levels. These results demonstrate that AR and SR have a combined effect in the FJHQ(SR)-induced antihyperglycemic action and elevating action on blood insulin level in STZ-diabetic mice.

Key words Boi-ogi-to, Stephania Radix, Astragali Radix, combined effect, STZ-diabetic model, antihyperglycemic effect, insulin release.

Abbreviations ALR, Atractylodes Lanceae Rhizoma, 蒼朮; AR, Astragali Radix, 黄耆; ELISA, enzyme-linked immunosorbent assay; FJHQ, Boi-ogi-to, Fang-ji-huang-qi-tang, 防已黄耆湯; GR, Glycyrrhizae Radix, 甘草; NIDDM, non-insulin-dependent diabetes mellitus; SCR, Sinomeni Caulis et Rhizoma, 防已; SR, Stephania Radix, 粉防已; STZ, streptozotocin; ZF, Zizyphi Fructus, 大棗; ZR, Zingiberis Rhizoma, 生姜.

Introduction

Kampo medicine, Boi-ogi-to (Fang-ji-huang-qi-tang: FJHQ), is one of the traditional prescriptions and has long been used clinically in the treatment of arthritis and edema in Japan and China. The prescription consists of six crude drugs: Sinomeni Caulis et Rhizoma (SCR), Astragali Radix (AR), Atractylodis Lanceae Rhizoma (ALR), Glycyrrhizae Radix (GR),

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Zingiberis Rhizoma (ZR) and Zizyphi Fructus (ZF) 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 similar diseases in Japan and China. FJHQ has been reported to inhibit the elevation of serum cholesterol and improve the ratio of visceral fat to somatic fat in diabetic patients with obesity.[1]

Diabetes mellitus is classified as "Shoukatsu" (Xiao Ke: Polyphagia and polyuria), and includes many symptoms such as polyurea, polydipsia, dry mouth and ketosis. The causes of diabetes mellitus are related with changes of life style such as overeating and lack of exercise. 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 months. Some investigators have recently reported that a single treatment of low doses of STZ in both neonatal and adult rodents results in characteristics of non-insulin-dependent diabetes mellitus (NIDDM)-like hyperglycemic state.[3-5] The STZ-diabetic model shows a mild hyperglycemia, impaired pancreatic function and insulin resistance. In addition, oral antidiabetic drugs containing tolubutamide and glibenclamide improve hyperglycemia and increase blood insulin level in STZ-diabetic rodents.[6-8] Since STZ-diabetic mice also have characteristics of NIDDM, we used STZ-induced diabetic ddY mice for this experiment.

We have previously reported that intraperitoneally administered FJHQ composed with SR [FJHQ (SR)] have antihyperglycemic action in STZ-diabetic mice to a greater extent than FJHQ composed with SCR[FJHQ(SCR)].[8] In the present study, the actions of FJHQ(SR) on hyperglycemia and blood insulin level in STZ-induced diabetic mice were compared with those of composed individual crude drugs, mixtures of crude drugs, FJHQ(SR) without individual crude drugs to study roles of constituent crude drugs in actions of FJHQ(SR). Time course of antihyperglycemic actions of FJHQ(SR) and related crude drugs was also compared with their actions on insulin levels of blood in STZ-diabetic mice to investigate mechanisms of FJHQ(SR) for its antihyperglycemic action.

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) were injected with a single dose (150 mg/kg, i.v.) of 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 in the control experiments. These mice were housed with the usual laboratory diet (PMI Lab Diet, Japan SLC, Shizuoka) and water *ad libitum* at 25-26°C with lights on from 7 a.m. to 7 p.m..

Preparation and administration of crude drugs: FJHQ(SR) is composed of the following six herbs: Stephania Radix (SR, 5 parts), Astragali Radix (AR; 5 parts), Atractylodis Lanceae Rhizoma (ALR; 3 parts), Glycyrrhizae Radix (GR; 1.5 parts), Zingiberis Rhizoma (ZR; 1 parts) and Zizyphi Fructus (ZF; 3 parts). FJHQ (SCR) is a component of Sinomeni Caulis et Rhizoma (SCR; 5 parts) as a substitute for SR. 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, AR from Neimeng gu, China during autumn, ALR from Hubei shen, China during autumn, GR from Xinbei bu, China during autumn, ZR from Guizhou shen, China during autumn, ZF from Henan shen, during autumn. FJHQ, its related combination and individual crude drugs were extracted in 6 volumes of distilled water at 96-98°C for 40 min with an automatic extractor "Torobi" (Tochimoto, Osaka, Japan). Water extracts were filtered through a mesh (No.42, Sanpo, Tokyo, Japan), lyophilized with a freeze-drier (DF-03G, ULVAC, Tokyo), and stored at 4°C. Dry weight yields (%) of extracts are shown in Table I. These extracts were suspended homogeneously in saline or saline containing 1% Avicel (Asahi Chemical Industry, Tokyo),[8] and administered orally (p.o.) through a gastric probe (0.2 ml/10 g body weight) or intraperitoneally (i.p.) (0.1 ml/10 g

Table I Extract yields of Fang-ji-huang-qi-tang (FJHQ), FJHQ without its included crude drugs and individual crude drugs.

Extract	Yield (w/w %)
Stephania Radix (SR)	5.7
Sinomeni Caulis et Rhizoma (SCR)	6.5
Astragali Radix (AR)	15.0
FJHQ (SR)	17.6
FJHQ (SCR)	16.2
FJHQ (SR)	
-SR	17.0
-AR	15.2
-Atractylodis Lanceae Rhizoma (ALR)	14.5
-Glycyrrhizae Radix (GR)	16.2
-Zizyphi Fructus (ZF)	11.0
-Zingiberis Rhizoma (ZR)	16.6

These extracts were prepared by being heated at 96-98°C in 6 volumes of distilled water for 40 min, and then filtered through a mesh and lyophilized with a freeze-drier.

body weight) into 3-14 hour-fasted mice, respectively.

Measurement of blood glucose and insulin levels : Blood samples were collected from orbital vein plexus of mice before, and 6 hours after i.p. administration of extracts, and before, and 6, 12 hours, 2, 4 and 6 days during p.o. administration of extracts once a day, respectively. Blood samples were then centrifuged at 3,000 rpm at 4°C for 20 min. Blood glucose and immunoreactive insulin levels of the supernatant were measured by the glucose oxidase method with a glucose B-test (Wako, Osaka) or Beckman glucose analyzer (type II, Beckman Coulter, Tokyo) and a mouse ELISA kit for insulin (Morinaga, Yokohama), respectively.

Statistical analyses : The data were expressed as means±S.E.M.. Data were evaluated by one-way ANOVA followed by the multiple range tests of Scheffe, or unpaired t-test. A value of $P<0.05$ was considered statistically significant.

Results

Anti-hyperglycemic effects of FJHQ(SR) by oral administration in STZ-diabetic mice

We have previously reported that FJHQ(SR) significantly decreases high blood glucose level of 20-hour-fasted STZ-diabetic mice 6 hours after its intraperitoneal administration.[8] FJHQ(SR) (160 mg/kg) significantly decreased high glucose level of 20 hour- and 26 hour-fasted diabetic mice compared with the saline group after 6 and 12 hours after its oral administration (Fig. 1, left). When FJHQ(SR) (160 mg/kg) was administered orally in fed diabetic mice once a day for 6 days, the blood glucose levels were reduced in a time-dependent manner. After 6 days, FJHQ(SR) fell 59 % of high glucose level in STZ-diabetic mice (Fig. 1 right).

However, FJHQ(SCR) (160 mg/kg) did not significantly decrease the blood glucose level under fasted or fed conditions compared with the saline group. These results demonstrate that FJHQ(SR) decreases high blood glucose level of STZ-diabetic mice by both oral and intraperitoneal administration. Intraperitoneal administration of FJHQ(SR) is useful to analyze antihyperglycemic action of FJHQ(SR) because accurate doses are administered into mice.

Elevating action of FJHQ(SR) by oral administration on blood insulin levels in STZ-diabetic mice

To investigate the mechanism of antihyperglycemic action of FJHQ(SR), immunoreactive insulin levels of blood were measured by ELISA kit under the condition of oral administration of FJHQ(SR) once a day for 6 days to fed diabetic mice (Fig. 2). Blood immunoreactive insulin level of STZ-diabetic mice was 322 pg/ml and decreased to 22 % of that of age-matched normal mice before administration of drug. FJHQ(SR) (160 mg/kg) significantly increased the insulin level at the maximal level on 2 days compared with the saline group and its effect was sustained plateau levels by 6 days (Fig. 2). The time course of action of FJHQ(SR) on elevation of insulin levels was earlier than that on glucose levels (Fig. 2). FJHQ (SCR) (160 mg/kg) only had a tendency to increase the insulin level but its effect was not significantly different from the saline group. These results demonstrate that the antihyperglycemic effect of FJHQ(SR) depends on the elevation of blood insulin level in the STZ-diabetic mice.

Combined effects of Stephania Radix and Astragali Radix in FJHQ(SR) on hyperglycemia in STZ-diabetic mice

The action of FJHQ(SR) was analyzed using

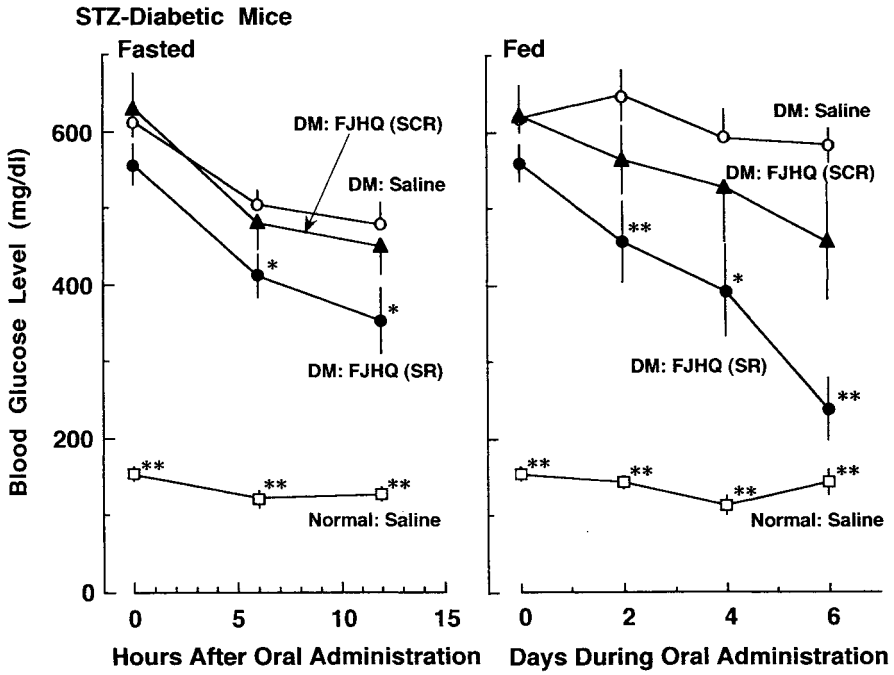

Fig. 1 Time-dependent antihyperglycemic effects of FJHQ(SR) (●), FJHQ(SCR) (▲) and saline (○, □) in STZ-diabetic (●, ▲, ○) and age-matched normal (□) fasted (left) and fed (right) mice. These crude drugs (160 mg/kg) were administered orally once into fasted mice (left) and once a day for 6 days into fed mice (right). The values are expressed as means ± S.E.M. of 8-13 data (left) and 14-19 data (right). *P<0.05, **P<0.01 : Significantly different from values with saline in diabetes mellitus (DM) on the corresponding hours (left) and days (right).

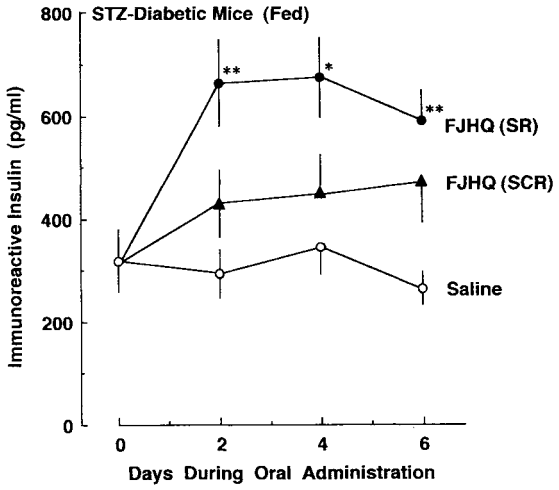

Fig. 2 Time-dependent elevating actions of FJHQ(SR) (●), FJHQ(SCR) (▲) and saline (○) on blood insulin level in STZ-diabetic fed mice. These drugs were orally administered into STZ-diabetic fed mice once a day for 6 days. The values are expressed as means±S.E.M. of 8-12 data. *P<0.05, **P<0.01 : Significantly different from values with saline on the corresponding days.

FJHQ without individual crude drugs, individual crude drugs and mixed crude drugs to investigate the combined effects of crude drugs in the action of FJHQ (SR). FJHQ without SR (48-160 mg/kg) and FJHQ without AR (160 mg/kg) significantly decreased the fall % of blood glucose compared with FJHQ(SR) did (Fig. 3, left). The effect of FJHQ(SCR) was also significantly weaker than that of FJHQ(SR). However, FJHQ without ALR, FJHQ without ZF, FJHQ without GR and FJHQ without ZR (48-160 mg/kg) showed a similar fall % of blood glucose level to that of FJHQ(SR). These results suggest that SR and AR may have a role in the antihyperglycemic action of FJHQ(SR).

Antihyperglycemic effects of individual SR and AR (16-160 mg/kg) were examined. SR dropped blood glucose level with a similar potency to that of FJHQ(SR), but AR did not affect it significantly in the STZ-diabetic mice (Fig. 3, right). These results propose the possibility that AR may have a combined

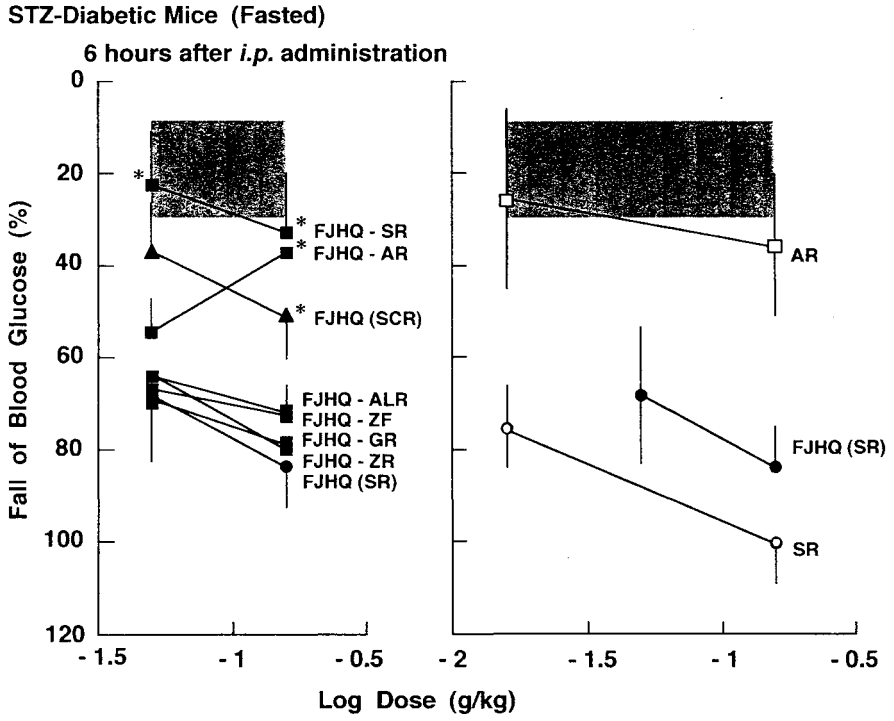

Fig. 3 Antihyperglycemic effects of FJHQ(SR) (●), FJHQ(SCR) (▲) and FJHQ without a composed individual crude drug (■) (left), and AR (□), SR (○) and FJHQ(SR) (●) (right) in STZ-diabetic mice. Blood glucose (BG) levels were measured before and 6 hours after intraperitoneal administration of drugs. The fall of BG (%) was calculated as [BG (before drug treatment) - BG (after drug treatment) / BG (before drug treatment) - 85] × 100. 85 are BG of 14 hour-fasted normal mice. The values were expressed as means±S.E.M. of 5-8 data (left) and 5-8 data (right). Shadowed columns represent ranges of standard errors of means of 7-8 data in saline alone. *P<0.05 : Significantly different from FJHQ(SR).

effect on the action of SR in the action of FJHQ(SR).

To prove the possible combined action of AR with SR, the antihyperglycemic effect of a mixture of SR and AR were directly compared with that of SR (Fig. 4). From the yield % of both crude drugs, the mixture of SR and AR was prepared as 1 : 3. SR (0.48-16 mg/kg) decreased blood glucose level in a dose-dependent manner. The same doses of AR did not affect it significantly. Dose-response curve of SR (0.12-4.0 mg/kg) mixed with 3 volumes of AR was sifted to the left side of the curve of SR alone. Fifty% inhibitory doses (ID50) of SR and SR mixed with AR with 95 % confident limit was calculated using net fall % values by drugs subtracted fall % values by saline. ID50 with 95 % confident limit of SR and SR mixed with AR were 7.75 mg/kg (3.52-17.06) and 0.99 mg/kg (0.35-2.75), respectively. These results demonstrate that the antihyperglycemic action of the mixture of SR and

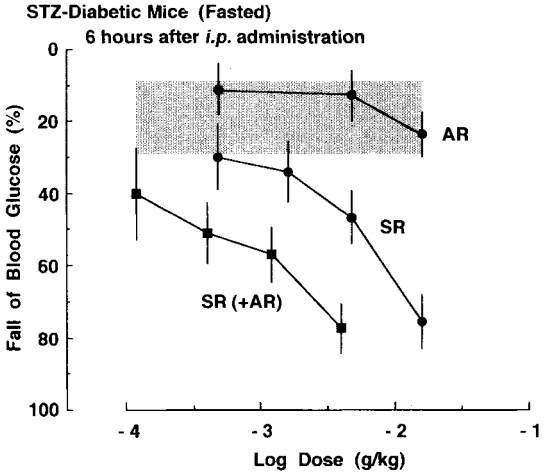

Fig. 4 Antihyperglycemic effects of AR, SR, and SR combined with AR [SR (+AR)] in STZ-diabetic mice. Blood glucose levels were measured before and 6 hours after intraperitoneal administration of AR, SR (●) and SR with AR (1 : 3) (■) into 14 hour-fasted diabetic mice. The values are represented means±S.E.M. of 6-14 data. Shadowed columns represent ranges of standard errors of means of 7 data in saline alone.

AR is 8-fold greater than that of SR alone, meaning that AR potentiates the action of SR in the hypoglycemic action of FJHQ in the diabetic mice.

Combined effects of Stephania Radix and Astragali Radix in the action of FJHQ(SR) on the increase in blood insulin levels in STZ-diabetic mice

Effects of SR, AR and mixture (1 : 3) of SR and AR on blood insulin levels were compared in the diabetic mice. SR(4.8 mg/kg) significantly increased the immunoreactive insulin level to 160 % of the level without drug 6 hours after intraperitoneal administration, but AR did not affect the insulin level (Fig. 5). The mixture of SR (4.8 mg/kg) with 3 volumes of AR significantly increased the insulin level to 289% of the level without drug. The effect of mixture is significantly greater than that of SR alone. The result of mixture of SR and AR on blood insulin levels were parallel with that on a high glucose level, meaning that AR potentiates the activity of SR on insulin release, followed by falling high blood glucose in the diabetic mice.

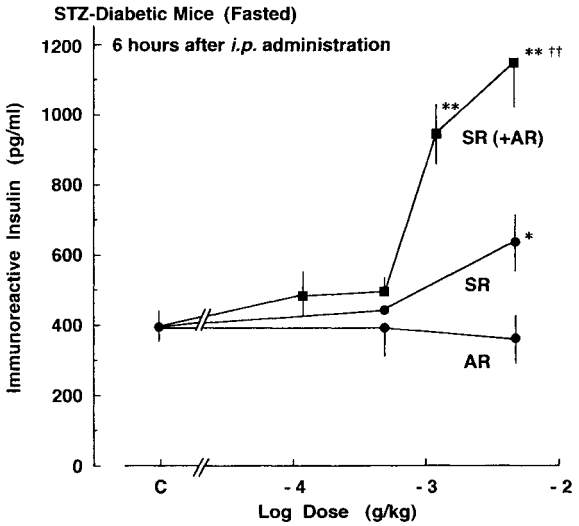

Fig. 5 Elevating actions of AR, SR and SR combined with AR [SR (+AR)] on blood insulin level in STZ-diabetic mice. The immunoreactive insulin levels were measured before and 6 hours after intraperitoneal administration of AR, SR (●) and SR with AR (1 : 3) (■) into 14 hour-fasted diabetic mice. The values are represented means± S.E.M. of 4-19 data. C represents the control without drug. * $P<0.05$, ** $P< 0.01$: Significantly different from control without drug. †† $P<0.01$: Significantly different from SR alone.

Discussion

We have previously reported that FJHQ(SR) dropped high glucose level of blood in STZ-diabetic mice to a greater extent than FJHQ(SCR) by their intraperitoneal administration.[8] The present study demonstrates that orally administrated FJHQ(SR) also significantly dropped high glucose level of blood in STZ-diabetic mice in an administration time-dependent manner. The time-course of maximal action of FJHQ(SR) on elevation of blood insulin levels occurred earlier than that of its antihyperglycemic action. These results indicate that the antihyperglycemic action of FJHQ(SR) is associated with its increase in blood insulin level released from the STZ-diabetic mice.

We have reported the strategy for the study of combined effects of crude drugs in actions of Kampo Hozai.[9-11] Using the strategy, the combined actions of crude drugs were investigated in the hypoglycemic action of FJHQ(SR). FJHQ(SR) without SR and FJHQ(SR) without AR significantly reduced the antihyperglycemic action of FJHQ(SR), but FJHQ(SR) without other individual crude drugs have similar actions as that of FJHQ(SR). SR had a similar antihyperglycemic action as FJHQ(SR), but AR did not have significant action compared with the saline group. These results mean that the action of SR is additive to the deficient action of FJHQ(SR) without SR in FJHQ(SR). However, since the action of AR is not additive to the deficient action of FJHQ(SR) without AR, a possible combined effect of AR with SR in FJHQ(SR) is suggested. To confirm the possible combined effect of AR and SR, effect of a mixture (1 : 3) of AR and SR was compared with that of SR. Since yields of AR and SR were 15.0 and 5.7 %, respectively (Table I), the mixture of these drugs was prepared as 1 : 3. The dose-response curve of mixture (4.8-160 mg/kg) on fall % of blood glucose sifted to the left side of that of SR alone. From their ID50 values of the mixture and SR alone, potency of the mixture is estimated to be 8-fold greater than that of SR alone. These results mean that AR has a potentiating effect on the action of SR in the antihyperglycemic action of FJHQ(SR) in STZ-diabetic mice.

The present study demonstrates that FJHQ(SR) without ALR, FJHQ(SR) without GR, FJHQ(SR) without ZR and FJHQ(SR) without ZF had similar antihyperglycemic action as that of FJHQ(SR) in STZ-diabetic mice. These results indicate that ALR, GR, ZR and ZF in FJHQ(SR) do not have a role for the initiation of antihyperglycemic action of FJHQ (SR), meaning that the combination of SR and AR in FJHQ(SR) is crucial to induce the antihyperglycemic action. In some prescription of FJHQ(SR), Rhizoma Atractylodis Macrocephalae is used as a substitute for ALR in Japan and China to remove moisture in body without diarrhea, compared with ALR. However, whether Rhizoma Atractylodis Macrocephalae has a role for the antihyperglycemic action of FJHQ(SR) or not is unclear.

To investigate mechanisms of potentiating effects of AR on the action of SR, their hyperinsulinemic effects were compared in blood of STZ-diabetic mice. SR, but not AR significantly increased blood immunoreactive insulin levels. The same dose of SR mixed with AR significantly increased it compared with SR alone. These results demonstrate that AR potentiates insulin release as well as the antihyperglycemic action of SR, meaning that the antihyperglycemic effect of FJHQ depends on the potentiating effect of AR on the insulin release of SR. We need more evidence for elucidation of mechanisms of insulin release of SR and potentiating action of AR on the insulin release in STZ-diabetic mice.

In conclusion, FJHQ (SR) improved hyperglycemia of the STZ-diabetic mice. The action of FJHQ(SR) is due to the combined effects of AR on the action of SR in FJHQ (SR). The antihyperglycemic action of FJHQ(SR) depended on its elevating action on immunoreactive insulin level in blood of STZ-diabetic mice. FJHQ(SR) has the substantial pharmacological basis of therapeutics in diabetes mellitus.

Acknowledgements

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和文抄録

粉防已を用いた防已黄耆湯（48-160 mg/kg/day）は，防已を用いた防已黄耆湯と比較して，ストレプトゾトシン(STZ)-誘発糖尿病 ddY マウスの高血糖値を有意に下降させた。防已黄耆湯の血糖下降作用は，腹腔内投与と経口投与のいずれにも見られ，血中へのインスリン遊離促進作用と平行した。各構成生薬の単味および混合物，方剤からの一味抜き方剤の血糖下降作用を防已黄耆湯の作用と比較した。防已黄耆湯から粉防已または黄耆を除くと，血糖下降作用は消失した。粉防已は単味で強力な血糖下降作用を示した。黄耆は，単味では作用を示さないが，粉防已の血糖下降作用を8倍も増強した。黄耆は，粉防已のインスリン遊離促進作用も増強した。以上の結果から，粉防已を用いた防已黄耆湯は，STZ-糖尿病態の高血糖値を改善し，その改善作用は，インスリン遊離促進作用に依存することを明らかにした。この防已黄耆湯の作用は，黄耆と粉防已の複合作用によって導かれると結論できた。

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