Antihyperglycemic effects of Mao-to (Ma-Huang-Tang), a Kampo formulation, in streptozotocin-induced diabetic mice

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Abstract

We investigated the effects of the Kampo formulations, Mao-to (MOT ; 麻黄湯, Ma-Huang-Tang), Shimbu-to (SBT ; 真武湯, Zhen-Wu-Tang), Ninjin-to (NJT ; 人参湯, Ren-Shen-Tang), Shigyaku-san (SGS ; 四逆散, Si-Ni-San), Keishi-to (KST ; 桂枝湯, Gui-Zhi-Tang) and Shimotsu-to (SMT ; 四物湯, Si-Wu-Tang), in streptozotocin (STZ)-induced diabetic mice. Among these 6 Kampo formulations, the oral ingestion of extract granules of MOT (1.0 g/kg/day) significantly decreased the blood glucose level in the STZ (200 mg/kg, i.p.)-induced diabetic mice. The antihyperglycemic effects were also observed after the oral ingestion of decoctions of MOT (400 mg/kg/day), Cinnamon Bark (CB, 70 mg/kg/day) and Ephedra Herb (EH, 160 mg/kg/day), which are contained in the formula of MOT. The blood glucose level in normal mice did not decrease after the oral ingestion of MOT, CB and EH. MOT or EH apparently improved the structure of islets damaged by STZ, possibly due to inducing hyperplasia of islet cells. The islet-protective effect of Kampo herbs has not yet been published. EH, however, had no effect after fasting, but tended to inhibit the increase in the blood level after injection with glucose (2 g/kg, i.p.). Thus, the antihyperglycemic effects of these formulae and herbs may be due to regulation of the blood glucose level in postprandial condition.

Key words Kampo formulae, Mao-to, hyperglycemia, streptozotocin, diabetic mice.

Abbreviations KST, Keishi-to, 桂枝湯, Gui-Zhi-Tang ; MOT, Mao-to, 麻黄湯, Ma-Huang-Tang ; NJT, Ninjin-to, 人参湯, Ren-Shen-Tang ; SBT, Shimbu-to, 真武湯, Zhen-Wu-Tang ; SGS, Shigyaku-san, 四逆散, Si-Ni-San ; SMT, Shimotsu-to, 四物湯, Si-Wu-Tang ; STZ, streptozotocin.

Introduction

The hypoglycemic effects of many kinds of Kampo herbs have already been reported.[1] In most of these studies, the hypoglycemic effects of the herb extracts or their fractions were examined in normal and/or experimentally induced hyperglycemic animals with intraperitoneal injection. The effects of orally administrated Kampo formulations on experimental diabetes have not been examined adequately.

We have focused on 6 Kampo formulations, Mao-to, Shimbu-to, Ninjin-to, Shigyaku-san, Keishi-to and Shimotsu-to, which differ in their utilities and contain relatively a few kind of herbs combination in formula, and have compared the pharmacological activity of these 6 formulations to examine the specificity of their actions.[2,3] In this study, we investigated the effects of these 6 Kampo formulations in STZ-induced diabetic mice.

Materials and Methods

Animals : BALB/cA Jcl male mice were obtained from Clea Japan, Inc. at 6 weeks of age. The animals were kept in plastic cages with wood shavings, 5-6 in

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each, and maintained in an animal room, which was air-conditioned (22-24°C), artificially illuminated and provided with standard commercial pellets and tap water *ad libitum*. At 7 weeks of age, the mice were intraperitoneally injected with 200 mg/kg of streptozotocin (STZ ; Sigma Chemical Co., St. Louis, USA) in 10 mM citrate buffer (pH 4.4) after 18 hours of fasting. On the fourth day after injecting the STZ, animals showing 200-500 mg/dl of blood glucose were divided into groups.

Crude drugs : Mao-to (MOT) and its component crude drugs (Uchida Wakan-Yaku Co.Ltd., Tokyo, Japan, the amount shown in Table I) were decocted with 600ml of boiled water until the volume was reduced to 300 ml. The supernatant of these extracts was filtered and stored at -20°C for the use. A part of the supernatant was lyophilized to check the yield.

Treatment : Experiment 1 : The experimental mice were given extract granules (Tsumura, Co., Table I) of Mao-to (MOT, TJ-27), Shimbu-to (SBT, TJ-30), Ninjin-to (NJT, TJ-32), Shigyaku-san (SGS, TJ-35), Keishi-to (KST, TJ-45) and Shimotsu-to (SMT, TJ-71) at the concentration of 1 % as drinking water from 4th day after the STZ injection.
Experiment 2 : The supernatant of the extract of MOT and its component crude drugs were diluted and given as drinking water from 4th day after the STZ injection . The dose of each herb was adjusted in the

Table I Prescription of Kampo formulae

Formulae	Herbs contained	Ratio (g)
Mao-to (TJ-27)	Glycyrrhiza Root	1.5
Formula ephedrae	Apricot Kernel	5.0
lot# : 240027010	Cinnamon Bark	4.0
	Ephedra Herb	5.0
Shimbu-to (TJ-30)	Peony Root	3.0
Formula	Ginger Rhizome	1.5
divinitatis nigrae	Atractylodes Lancea Rhizome	3.0
lot# : 250030010	Hoelen	4.0
	Heat Processed	
	Acnite Tuber Powder	0.5
Ninjin-to (TJ-32)	Atractylodes Lancea Rhizome	3.0
Formula ginseng	Glycyrrhiza Root	3.0
lot# : 920032001P0	Ginseng Root	3.0
	Dried Ginger Rhizome	3.0
Shigyaku-san (TJ-35)	Bupleurum Root	5.0
Pulvis quadri-frigorium	Peony Root	4.0
lot# : 250035010	Immature Orange	2.0
	Glycyrrhiza Root	1.5
Keishi-to (TJ-45)	Peony Root	4.0
Formula cinnamomi	Ginger Rhizome	1.5
lot# : 230045010	Cinnamon Bark	4.0
	Glycyrrhiza Root	2.0
	Jujube Fruit	4.0
Shimotsu-to (TJ-71)	Peony Root	3.0
Formula quadri-	Rehmaninia Root	3.0
medicamentorum	Japanese Angelica Root	3.0
lot# : 250071020	Cnidium Rhizome	3.0

consistency with the proportion of each herb in MOT (10 times of human dose). To make sure that same doses were administered for the period of the whole experiment, the water intake was checked every 2 days by measuring the differences in the weight of water bottles after 2 consecutive days in each cage. Based on the above measurement, the dilution of samples was determined every 2 days.

Experiment 3: The same samples were given to BALB/c normal mice at the same dose as in Experiment 2. In all experiments, the control mice were provided with tap water *ad libitum* throughout the experiment.

Blood glucose levels: Whole blood samples were collected from tail veins at 9:00-10:00 on days 0, 3, 7 and 14 of treatment of the formulations or herbs. Immediately after bleeding, the blood glucose levels were determined by the glucose dehydrogenase method on a portable glucometer (Advantage, Yamanouchi, Tokyo, Japan).

Histological observation: After 14 days of treatment, the mice were killed by decapitation under light ether anesthesia. Whole pancreatic tissue in 10% buffered formalin was embedded in paraffin and sliced at 6 μm. A section, randomly selected from each sample, was stained with haematoxylin-eosin. A representative section from each group was analyzed for number and area of islets, and area of whole section.

Serum insulin levels: At autopsy, the blood was collected from the trunk, allowed to clot for 1 hour and centrifuged at 1000 xg for 15 min at 4°C. Serum was stored at -20°C for assay. The level of insulin was determined by ELISA Insulin kit (Morinaga, Yokohama, Japan).

Glucose tolerance test: STZ-induced diabetic mice were administered with EH for 2 weeks (see Experiment 2) and were fasted for 18 hours before the glucose loading. To determine only pancreatic function and to exclude the involvement of various metabolic procedures, glucose (2 g/kg) was intraperitoneally injected. The blood glucose levels were measured just before the glucose loading and at 30, 60 and 120 minutes later.

Statistics: The difference in each parameter among groups was evaluated by Student's *t*-test.

Results

The effects of 6 Kampo formulations on the blood glucose level in diabetic mice (Experiment 1)

As shown in Fig. 1, the blood glucose level (257±26 mg/dl) in the MOT-treated mice was significantly

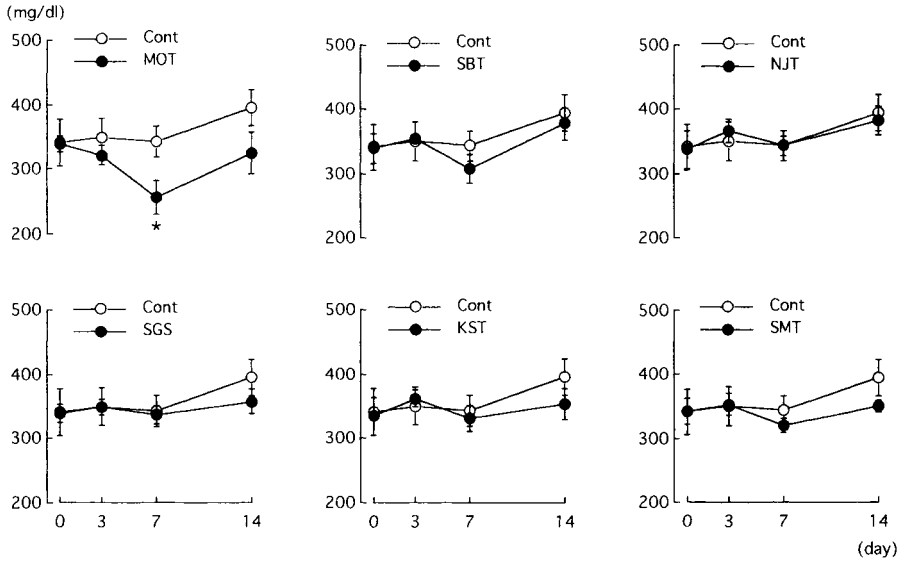

Fig. 1 Effects of Kampo formulations on the blood glucose level in STZ-induced diabetic mice (Mean±S.E.M.). Significantly different from the controls $*p<0.05$. Cont, diabetic controls; MOT, Mao-to (麻黄湯); SBT, Shimbu-to (真武湯); NJT, Ninjin-to (人参湯); SGS, Shigyaku-san (四逆散); KST, Keishi-to (桂枝湯); SMT, Shimotsu-to (四物湯).

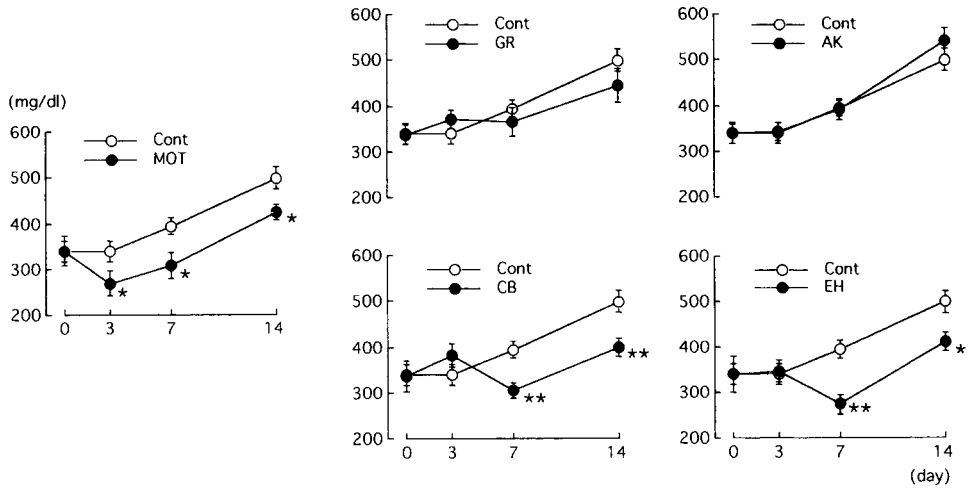

Fig. 2 Effects of MOT and its component herbs on the blood glucose level in STZ-induced diabetic mice (Mean±S.E.M.). Significantly different from the controls $*p<0.05$ / $**p<0.01$. Cont, diabetic controls ; MOT, Mao-to (麻黄湯) ; GR, Glycyrrhiza Root (甘草) ; AK, Apricot Kernel (杏仁) ; CB, Cinnamon Bark (桂枝) ; EH, Ephedra Herb (麻黄).

lower than in the control (343±24 mg/dl), on day 7 of treatment. This trend was also observed on day 14 (325±33 *v.s.* 395±28), but, the difference was not statistically significant. On the other hand, no significant differences were observed between the control and the other experimental groups. Doses (g/kg/day) of Kampo medicines calculated from water intake were 1.0, 1.8, 1.7, 1.8, 1.8 and 1.4 for MOT, SBT, NJT, SGS, KST and SMT, respectively.

The effects of Mao-to and its component herbs on the blood glucose level in diabetic mice (Experiment 2)

Since a significant decrease in blood glucose was observed in the group treated with extract of MOT, the effects of water extract of MOT and its component herbs on the same model were examined. As shown in Fig. 2, the blood glucose in MOT-treated mice was significantly lower than in the controls on days 3, 7 and 14. Decreased blood glucose levels were also observed in the CB- and EH-treated groups on days 7 and 14. The blood glucose levels were not significantly changed by the GR- and AK-treatment throughout the experiment. Doses (mg/kg/day) of extract solutions calculated from water intake were 400, 90, 120, 70 and 160 for MOT, GR, AK, CB and EH, respectively.

The effects of Mao-to and its component herbs on the blood glucose level in normal mice (Experiment 3)

The effects of MOT, CB and EH, which signifi-

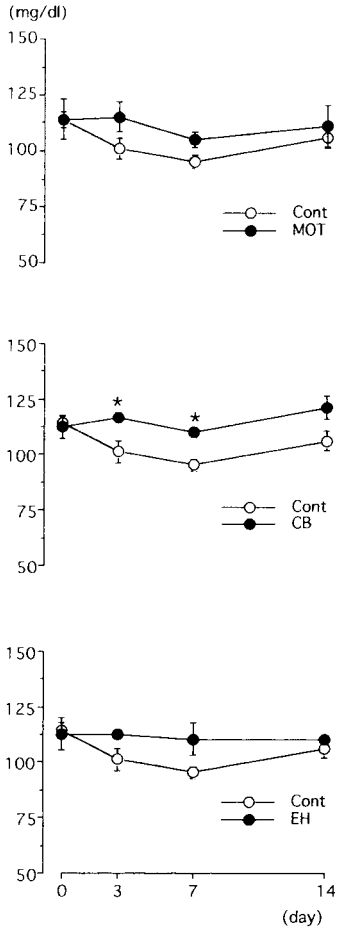

Fig. 3 Effects of MOT, CB and EH on the blood glucose level in normal BALB/c mice (Mean±S.E.M.). Significantly different from the controls at $*p<0.05$. MOT, Mao-to (麻黄湯) ; CB, Cinnamon Bark (桂枝) ; EH, Ephedra Herb (麻黄).

cantly decreased the blood glucose in STZ-induced diabetic mice, on non-diabetic normal mice were examined. In all experimental groups, the blood glucose levels did not decrease compared to the controls, and in the CB-treated group, they were significantly higher than in the controls. (Fig. 3).

Histological observation

In the diabetic controls, a severe degenerative

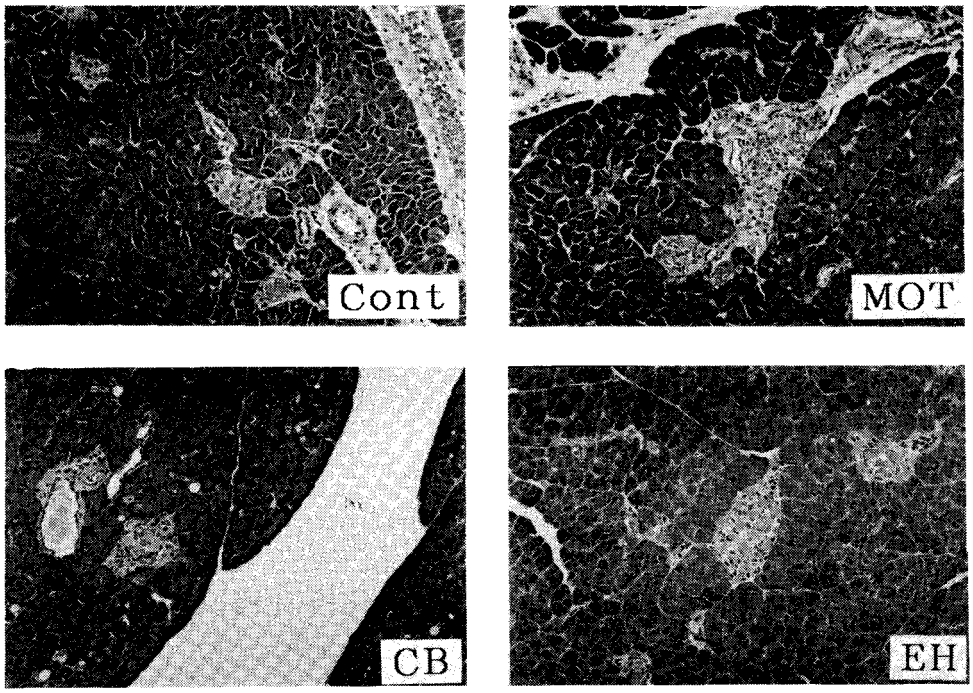

Fig. 4 Effects of MOT, CB and EH on the structure of pancreatic tissue in STZ-induced diabetic mice (Haematoxylin/eosin, ×200). Cont, Diabetic controls ; MOT, Mao-to (麻黄湯) ; CB, Cinnamon Bark (桂枝) ; EH, Ephedra Herb (麻黄).

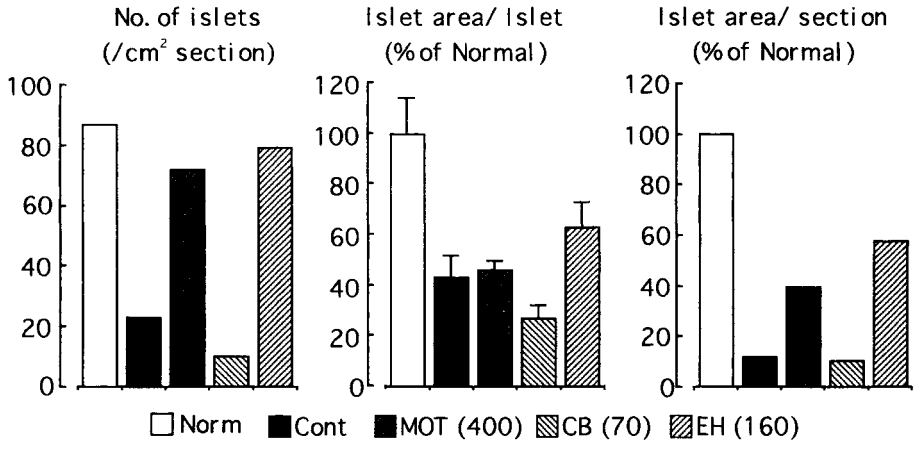

Fig. 5 Effects of MOT, CB and EH on the structure of pancreatic islets in STZ-induced diabetic mice. Dose (mg/kg/day) of each extract calculated from water intake is given in parentheses. Norm, Normal controls ; Cont, Diabetic controls ; Mao-to (麻黄湯) ; CB, Cinnamon Bark (桂枝) ; EH, Ephedra Herb (麻黄).

atrophy in the structure (Fig. 4, upper left) and a decrease in the number of the pancreatic islets were observed. In MOT- (upper right) and EH- (lower right) treated mice, less atrophy and a larger number of islets were observed than in the diabetic controls. The number of islets per sq. cm section, the area of each islet and islet area per section were lower in the diabetic controls than in the normal controls (Fig. 5). The number of the islets per sq. cm section and islet area per section were apparently improved by MOT- and EH-treatment. Area of each islet was not significantly changed.

Serum level of insulin (Fig. 6)

No significant differences were observed between the diabetic controls and the MOT-treated group on serum insulin level in either Experiment 1 or 2. The serum insulin level tended to be higher in the CB- and EH-treated groups than in the controls, but, the difference was not statistically significant.

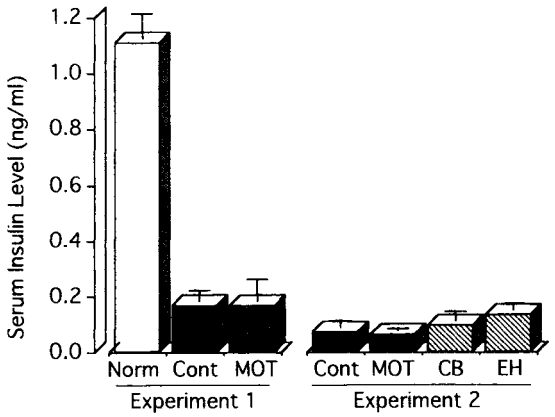

Fig. 6 Effects of MOT, CB and EH on the serum insulin level in STZ-induced diabetic mice (Mean±S.E.M.). Norm, Normal controls ; Cont, Diabetic controls ; MOT, Mao-to (麻黄湯) ; CB, Cinnamon Bark (桂枝) ; EH, Ephedra Herb (麻黄).

Glucose tolerance test (Fig. 7)

The blood glucose level was apparently lowered by EH-treatment for 2 weeks, but, the difference was not observed after the fasting for 18 hours. In the diabetic controls, the blood glucose level increased on the process of time until 120 minutes after the injection of glucose, while, the levels in EH-treated group stopped increasing from 30 minutes after the glucose-injection, showing lower level of glucose than in the diabetic controls with no statistical significance.

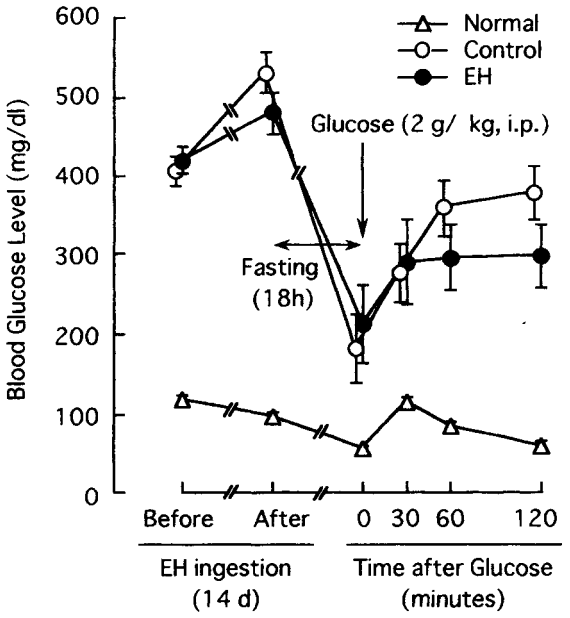

Fig. 7 Effects of Ephedra Herb (EH) on glucose (2 g/kg, i.p.) tolerance in STZ-induced diabetic mice (Mean±S.E.M.). EH, Ephedra Herb (麻黄, 160 mg/kg/day).

Discussion

The present results suggest that MOT shows a significant antihyperglycemic effects in STZ-induced diabetic mice. In the experiment in which the effects of the extract of 6 basic Kampo formulations were compared, only MOT significantly decreased the blood glucose level. The antihyperglycemic effects were observed, not only in extract granules but also in a decoction of MOT. These results suggest that the oral ingestion of MOT has reliable antihyperglycemic effects.

The effects of GR, AK, CB and EH, herbs contained in the formulaion of MOT, were examined and among them CB and EH showed the significant antihyperglycemic effects. The hypoglycemic effect of an aqueous methanol extract of EH and its component, glycans has been reported[4] ; however, these activities were examined in normal and hyperglycemic animals which had been intraperitoneally injected with samples. In addition, the antihyperglycemic effect of MOT was observed from day 3 of

treatment, while, the effects of CB and EH were observed from day 7. These results suggest that the superior effect of MOT may be due to the added effects of CB and EH.

On the other hand, the blood glucose in normal mice was not decreased by MOT, CB and EH, which were shown to have antihyperglycemic effects in STZ-induced diabetic mice. These data suggest that the effects of these formulation and herbs are different from those of oral hypoglycemic sulfonylureas, which lowers blood glucose levels in normal or mild diabetic animal.

The structure of islets destroyed by STZ was apparently improved by MOT and EH, possibly due to induced hyperplasia of islet cells. Thus this effect may have contributed to the antihyperglycemic effects of MOT and EH. The hypoglycemic effects of many kinds of Kampo herbs have been reported, but no islet-protective effect has been published. On the other hand, the increases in serum insulin levels were not enough to explain the islet-protective effects of these formulation and herb. However, serum insulin levels were determined under conditions in which intake of calories was not controlled. EH lowered the blood glucose levels when diabetic mice were allowed free access to food, but did not effect them after fasting and again tended to inhibit the increase after injection with glucose. Thus it is suggested that the antihyperglycemic effects of these formulation and herb may be due to regulation of the blood glucose, which increase after food intake.

Konno *et al.*[4] reported that administration of an aqueous methanol extract of EH and glycans isolated from EH exhibited the significant hypoglycemic effects in normal and alloxan-induced hyperglycemic mice. These results were obtained from different experimental protocols and suggest a different mechanism of actions from ours. At present, we are investigating the effect of the fraction of extracts of EH, including ephedrine, a major active component of EH, as well as long-term oral administration of EH or its fractions.

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和文抄録

Streptozotocin (STZ, 200 mg/kg) を投与することにより糖尿病を誘発したBALB/c (雄, 7週齢) マウスの高血糖に対する麻黄湯, 真武湯, 人参湯, 四逆散, 桂枝湯および四物湯の影響を検討した。これら6種の漢方方剤のうち麻黄湯エキス顆粒 (1.0 g/kg/day, 飲水として自由摂取) が高血糖を有意に抑制した。抗高血糖作用は生薬から煎出した麻黄湯 (400 mg/kg/day) や麻黄湯の構成生薬である桂枝 (70 mg/kg/day) および麻黄 (160 mg/kg/day) にも認められた。麻黄湯, 桂枝および麻黄は正常マウスの血糖を低下させることはなかった。麻黄湯および麻黄はSTZによる膵島の障害を明らかに改善したが, 漢方薬の膵島保護作用に関する報告はまだない。麻黄は空腹時血糖には影響しなかったが, グルコース (2 g/kg, i.p.) 負荷後の血糖の上昇を抑制する傾向があった。よって, 麻黄湯および麻黄は血糖の上昇を抑制することによって高血糖を抑制することが示唆された。

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