Antidiabetic effect of Chibaku-rokumi-gan in KK-Ay mice

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Abstract

The antidiabetic effect of Chibaku-rokumi-gan (知柏六味丸; CRG) (TJS-169) was investigated in KK-Ay mice, one of the animal models of non-insulin dependent diabetic mellitus (NIDDM). CRG (1650 mg/kg body weight) reduced the blood glucose of KK-Ay mice from 488±27 mg/dl to 288±36 mg/dl 10 hours after single oral administration ($p<0.001$), and also lowered the plasma insulin from 415±8 to 203±53 μU/ml ($p<0.05$). CRG-treated KK-Ay mice showed a significant decrease in blood glucose 7-10 weeks after administration. These results support that CRG improve glucose metabolism by reducing insulin resistance. Therefore, CRG may be useful for treatment of NIDDM.

Key words antidiabetic effect, Chibaku-rokumi-gan, TJS-169, KK-Ay mice, insulin resistance.

Introduction

Chibaku-rokumi-gan (知柏六味丸; CRG) has been used as a traditional medicine for diabetes (polyuria and polydipsia).[1] Recently, CRG also was used for systemic lupus erythematosis.[2] However, there is no experimental evidence of improving hyperglycemia. In the present study, we investigated the antidiabetic effect of CRG using KK-Ay diabetic mice, one of the animal models of non-insulin dependent diabetes mellitus (NIDDM).

Materials and Methods

Materials : The traditional Chinese preparation, Chibaku-rokumi-gan (CRG) was obtained from Tsumura Co., Tokyo Japan. The constituents of CRG was indicated including 8 raw medicines, Rehmanniae Radix 6.0 g, Anemarrhenae Rhizoma 3.0 g, Hoelen 4.0 g, Alismatis Rhizoma 4.0 g, Dioscoreae Rhizoma 4.0 g, Moutan Cortex 4.0 g, Phellodendri Cortex 2.0 g. CRG (crude powder extract) contains apray-dried aqueous extracts of 8 crude drugs as a mixture. The yield was 31.89 %. On single administration, CRG was dissolved in distilled water. CRG diets contain 2 % CRG extract.

Animals : Adult male ddY mice (SLC, Shizuoka, Japan) weighing 22-25 g were used. The mice were housed in an air-conditioned room at 22±2°C with a 12 hours light and 12 hours dark cycle. The animals were kept in the experimental animal room for 7 days with free access to food and water. For the determination of blood glucose levels, blood samples were withdrawn from the cavernous sinus with a capillary.

KK-Ay mice (Clea, Tokyo, Japan), 12 weeks old, were used. KK-Ay mice with blood glucose level above 300 mg/dl considered to be diabetic were used in this study. Four to seven animals were used for each group.

Drug : Tolbutamide was obtained by Sigma (Tokyo, Japan).

Determination of blood glucose and insulin : Blood glucose levels in both normal and diabetic animals were determined by glucose oxidase method[2] and plasma insulin was measured by a GLAZYME Insulin-EIA TEST.[3] All the data were expressed as means±

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S.E.M. 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

The mean blood glucose levels of KK-Ay mice at various time intervals after single oral administration of CRG are shown in Fig. 1. CRG (825 mg/kg, p.o.) lowered blood glucose from the basal value of 465±24 mg/dl to 318±32 mg/dl ($p<0.01$), 7 hours after the administration. After 10 hours, blood glucose also was significantly decreased ($p<0.01$). CRG (1650 mg/kg) also lowered blood glucose level at 7 hours (from 488 ±27 to 352±40, $p<0.05$) and 10 hours (288±36, $p<0.001$), CRG (1650 mg/kg)-treated mice showed a significant decrease in plasma insulin at 10 hours when compared with the basal values ($p<0.05$). (Fig. 2). Insulin (positive control) at a dose of 5 U/kg showed lower blood glucose levels over the period 2 to 4 hours after the administration.

The effects of oral administration of CRG (1650 mg/kg) on the blood glucose of normal mice are shown in Fig. 3. No differences in blood glucose were observed between the levels at times 2, 4, 7, and 10 hours after administration, when compared with the basal values (at 0 hour) in control mice. Tolubutamide (a known sulfonylurea hypoglycemic agent) at a dose of 50 mg/kg showed lower blood glucose levels over the period 2 to 10 hours after the administration.

The antidiabetic effect of CRG diet (containing 2 % CRG) was shown in Fig. 4. CRG-treated KK-Ay mice showed a significant decrease in blood glucose

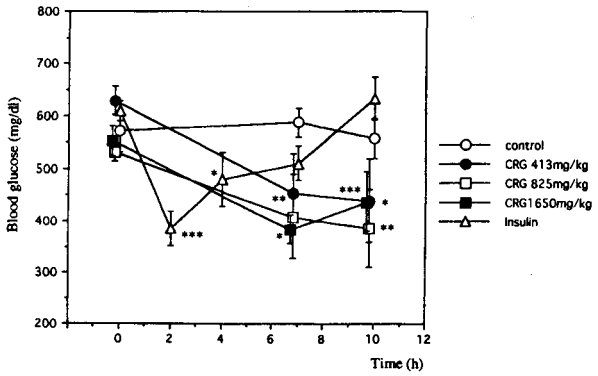

Fig. 1 Effects of Chibaku-rokumi-gan (CRG) on blood glucose in KK-Ay mice (Single administration).
CRG (413, 825, 1650 mg/kg) and insulin (5 U/kg) were administered to the mice and after 7 and 10 h, blood samples were taken for glucose determinations. Each value represents the mean±S.E.M. from 4-7 mice. Significantly different from control, $*p<0.05$, $**p<0.01$, $***p<0.001$.

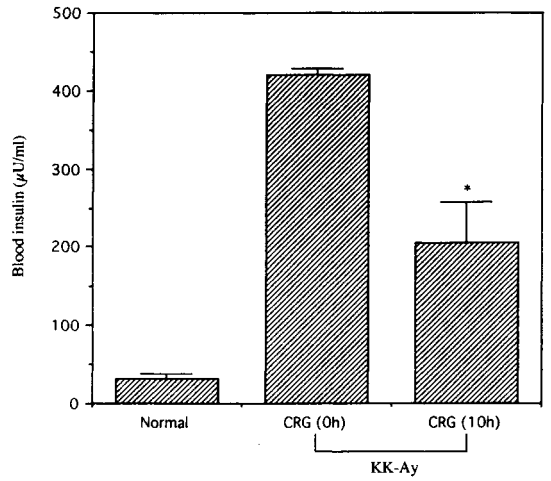

Fig. 2 Effects of Chibaku-rokumi-gan (CRG) on plasma insulin in KK-Ay mice (10 h).
CRG (1650 mg/kg) was administered to the mice and 10 h after single administration, blood samples were taken for insulin determinations. Each value represents the mean± S.E.M. from 5 mice. Significantly different from control, $*p<0.05$.

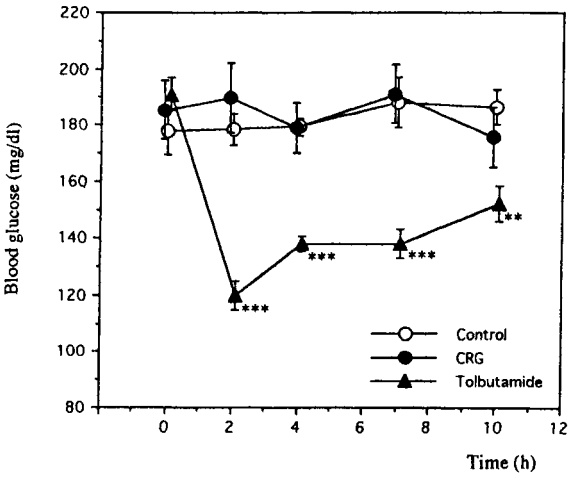

Fig. 3 Effects of Chibaku-rokumi-gan (CRG) on blood glucose in ddY mice.
CRG (1650 mg / kg) and tolbutamide (50 mg / kg) was administered orally to the mice and after 2, 4, 7 and 10 h, blood samples were taken for glucose determinations. Each value represents the mean±S.E.M. from 6 mice. Significantly different from control, $**p<0.01$, $***p<0.001$.

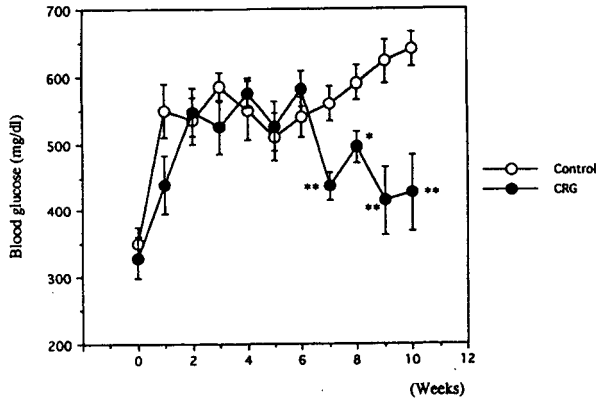

Fig. 4 Effects of Chibaku-rokumi-gan (CRG) on blood glucose in KK-Ay mice (long administration).
CRG diet (containing 2 % CRG) was administered to the mice and several weeks after the administration, blood samples were taken for glucose determinations. Each value represents the mean±S.E.M. from 8 mice. Significantly different from control, $*p<0.05$, $**p<0.01$, $***p<0.001$.

7-10 weeks after the administration when compared with control.

Discussion

The KK mouse strain[4] develops moderate degrees of obesity and diabetes that are especially apparent in animals>5 mo old and/or when fed a high-caloric diet.[5] The lethal yellow agouti gene (Ay) into KK mice has resulted in a congenic lethal yellow obese KK mouse strain, KK-Ay mice,[6] which are characterized by severe obesity, hyperinsulinemia and insulin resistance, features of NIDDM.[7] Both KK and KK-Ay mice have provided a useful model system to study the pathogenesis, therapy, and prevention of obesity and diabetes.[8]

The present study clearly shows that CRG produces consistent hypoglycemic effects in KK-Ay mice. CRG-treated KK-Ay mice decreased the blood glucose, indicating that CRG can lessen the insulin resistance. This action was at 7-10 h. This finding suggested that CRG acted after the metabolic process. CRG had no effect on blood glucose levels in normal mice. Furthermore, CRG showed a significant decrease on insulin level in KK-Ay mice. From these findings, hypoglycemic effect of CRG needs the states of hyperinsulinemia in order to act. CRG had an antidiabetic effect in single and long administration. It is suggested that single administration of CRG led to the result of its long administration.

Further studies will be needed to elucidate the mechanism of these effects. These results suggest the validity of clinical use of Chibaku-rokumi-gan in the treatment of diabetes mellitus, especially in NIDDM.

和文抄録

知柏六味丸(CRG) (TJS-168)の抗糖尿病作用を KK-Ay(インスリン非依存型糖尿病)で検討した。CRG 1650 mg/kg (ヒトの投与量の 10 倍量) は投与後 10 時間に血糖値 488±27 から 288±36 まで低下させた ($p<0.001$)。また，血漿インスリン値も 415±8 から 202±53 まで低下させた ($p<0.05$)。しかし，正常マウス (ddY) の血糖値には変化を与えなかった。

CRG はまた長期投与において KK-Ay マウスの血糖値を 7 週目より低下させた。これらの結果より CRG の血糖低下作用は末梢のインスリン抵抗性を緩和し，高インスリン血漿を改善するものと考えられる。それゆえ，CRG のインスリン非依存型糖尿病への有効性が示唆された。

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