Hypoglycemic Effect of the Rhizomes of *Ophiopogonis Tuber* in Normal and Diabetic Mice

Mami KAKO,^a Toshihiro MIURA,*^a Masaru USAMI,^a Atsushi KATO,^b and Seizou KADOWAKI^a

Suzuka University of Medical Science and Technology,^a Kishioka 1001-1, Suzuka, Mie 510-02, Japan and Kobe Pharmaceutical University,^b 4-19-1, Motoyamakita-machi, Higashinada-ku, Kobe, Hyogo 658, Japan.

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The hypoglycemic effect of the rhizomes of *Ophiopogonis Tuber* (Liliaceae) was investigated in normal and streptozotocin-induced diabetic mice. The *n*-butanol extract of rhizomes of *Ophiopogonis Tuber* (BM) (100 mg/kg) reduced the blood glucose of normal mice from 201 ± 13 to 151 ± 7 mg/100 ml 4 h after intraperitoneal administration ($p < 0.05$), and also significantly lowered the blood glucose of streptozotocin-induced diabetic mice from 590 ± 28 to 470 ± 37 mg/100 ml under similar conditions ($p < 0.05$). BM also tended to suppress epinephrine-induced hyperglycemia in mice. We concluded that the hypoglycemic effect of BM does not alter the insulin concentration.

Key words hypoglycemic effect; *Ophiopogon Tuber*; Liliaceae; oriental traditional medicine; diabetes; epinephrine

The rhizomes of *Ophiopogon japonicus* (Liliaceae) (bakumondo in oriental medicine) have been used as a traditional medicine for diabetes (polyuria and polydipsia) and weak constitution. Kohda reported that water extract-treated rabbit reduced the blood glucose of normal and alloxan-induced diabetic rabbit after oral administration.¹⁾ The constituents of *Ophiopogonis Tuber* have been chemically determined,²⁾ including by the use of some steroidal glycosides. However, there is no clear experimental evidence of the hypoglycemic action of these materials. The purpose of this study was to examine the hypoglycemic effect of the rhizomes of *Ophiopogonis Tuber*.

MATERIALS AND METHODS

Rhizomes of *Ophiopogonis Tuber* from a market in China and supplied by Tochimoto Tenkaido Co., Ltd. (Japan) were used. Five hundred grams of the rhizomes were extracted with 2 l of methanol (65 °C, 2 h 4 times). The methanol extracts were lyophilized (24 g) and stored at 4 °C until use. The *n*-butanol fraction (BM) was separated by a conventional method. The methanol extract was partitioned between *n*-butanol and water, and the *n*-butanol layer was evaporated to dryness (2.5 g).

Animals Adult male ddY mice weighing 22–25 g were used. The mice were housed in an air-conditioned room at 22 ± 2 °C with a 12 h light–12 h dark cycle. The animals were kept in the experimental animal room for 7 d 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.

The animals were divided into two groups. One was injected intravenously with 150 mg/kg body weight of streptozotocin (STZ) freshly dissolved in citrate buffer pH 4.5, and the other group was given buffer alone and used as a control. Eight days after injection of STZ, the blood glucose levels of all the mice were determined. Those with a blood glucose level above 300 mg/100 ml were considered to be diabetic and were used in the study. The hypoglycemic effect of BM was compared with that of tolbutamide and insulin in normal and STZ-induced

diabetic mice, respectively. Four to six animals were used for each group. BM-treated mice were injected with 100 mg/kg body weight of the *n*-butanol fraction dissolved in 10 ml of distilled water.

Oral Glucose Tolerance Test After overnight (18 h) fasting, the mice were intraperitoneally given BM and, 4 h later, the glucose (2 g/kg body weight) solution was administered orally. Blood samples were collected before the administration of the glucose and 0.5, 1, and 2 h later.

Epinephrine-Induced Hyperglycemic Mice The adult ddY mice were given BM intraperitoneally and, 4 h later, the epinephrine (0.6 mg/kg body weight) solution was also administered intraperitoneally. Blood samples were collected 1 h after the administration of epinephrine.

Determination of Blood Glucose and Insulin Blood glucose levels in both normal and diabetic animals were determined by glucose oxidase method³⁾ and serum insulin was measured by enzyme method.⁴⁾ All the data were expressed as means $\pm$ S.E. and Student's *t* test was used for the statistical analysis. The values were viewed as significantly different when the *p* value was less than 0.05.

To the liver (1.0 g), 30% KOH solution (2.0 ml) was added and the mixture was boiled for 30 min. Ice-cold 95% ethanol (4.0 ml) was then added and kept for 30 min at 4 °C. After two further treatments with ethanol, the combined total precipitate was dissolved in water (1.0 ml) and the glycogen content measured by the anthrone–H₂SO₄ method.⁵⁾

RESULTS

Effect of BM on Blood Glucose and Insulin in Normal Mice The mean blood glucose levels of mice at various time intervals after intraperitoneal administration of BM are shown in Fig. 1. These levels were compared with the values in control mice administered saline alone and also with those in animals receiving 50 mg/kg body weight of tolbutamide, a known sulfonylurea hypoglycemic agent (Fig. 1). The hypoglycemic effect of BM was dose-dependent. The serum insulin levels in BM (100 mg/kg)-

* To whom correspondence should be addressed.

Fig. 1. Effect of BM on Blood Glucose Level in Normal Mice

Each value represents the mean $\pm$ S.E. from 4–5 mice. Significantly different from prevalue, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. —□—, control; —◆—, BM 4 mg/kg; —■—, BM 20 mg/kg; —◇—, BM 100 mg/kg; —■—, tolbutamide 50 mg/kg.

Fig. 2. Effect of BM on Blood Glucose in STZ-Induced Diabetic Mice

Each value represents the mean $\pm$ S.E. from 3–6 mice. Significantly different from prevalue, * $p < 0.05$, *** $p < 0.001$. —□—, control; —◆—, BM 20 mg/kg; —■—, BM 100 mg/kg; —◇—, Insulin 5 U/kg.

treated mice did not change at 4h, while the tolbutamide-treated mice showed lower blood glucose levels during the period 2 to 7h after administration.

Effect of BM on Blood Glucose in STZ-Induced Diabetic Mice The hypoglycemic effects of BM and insulin on the blood glucose of STZ-induced diabetic mice are shown in Fig. 2. No differences in blood glucose were observed between the levels at times 4 and 7h after administration, when compared with the basal values (at 0h) in control mice. However, BM 100 mg/kg-treated mice showed a significant decrease in blood glucose after 4h when compared with the basal values ($p < 0.05$). Insulin-treated mice (5 U/kg body weight) exhibited a significant decrease in blood glucose at 2h when compared with the basal values ($p < 0.05$).

Oral Glucose Tolerance Test Intraperitoneally BM-treated animals (100 mg/kg body weight) showed no decrease in blood glucose when compared with controls.

Effect of BM on Blood Glucose in Epinephrine-Induced Hyperglycemic Mice The effect of BM injected intraperitoneally on epinephrine-induced hyperglycemia is shown Fig. 3. BM-treated animals (100 mg/kg body weight) showed lower blood glucose levels than the

Fig. 3. Effect of BM on Blood Glucose in Epinephrine-Induced Hyperglycemic Mice

Each value represents the mean $\pm$ S.E. from 5 mice. Significantly different from control, ** $p < 0.01$.

Fig. 4. Effect of BM on Glycogen Content in Epinephrine-Induced Hyperglycemic Mice

Each value represents the mean $\pm$ S.E. from 5–6 mice. Significantly different from control, * $p < 0.05$.

untreated group ($p < 0.01$). They also showed a significant increase of glycogen level in the liver ($p < 0.05$, Fig. 4).

DISCUSSION

The present study clearly showed that the *n*-butanol extract of the rhizomes of *Ophiopogonis Tuber* (BM) produces consistent hypoglycemic effects in normal mice. In addition, we examined the therapeutic effect of BM on hyperglycemia in STZ-induced diabetes in mice, one of the animal models of insulin-dependent diabetes mellitus (IDDM). After treatment of the mice with BM, the resulting hypoglycemia was observed without any change in serum insulin.

The blood glucose level lowered continuously for 7h following administration in normal mice treated with BM. STZ-induced mice did not show a significant decrease in blood glucose level at 7h when treated with BM (100 mg/kg), thus suggesting a relation with insulin concentration. No difference in blood glucose levels was observed in fasted mice. Synthetic drugs (insulin, tolbutamide) usually have hypoglycemia as one of their side effects, this finding indicates that BM is a useful drug. BM-treated mice suppress epinephrine-induced hypergly-

cemia. It seems likely that the hypoglycemic effect of BM may be the result of its decreasing the hepatic glucose output in both normal and epinephrine-induced hyperglycemic mice.

Further studies are needed to clarify the details. These results suggest the validity of clinical use of *Ophiopogonis* Tuber in the treatment of diabetes mellitus.

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