Hypoglycemic Activity of Polygonati Rhizoma in Normal and Diabetic Mice

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The hypoglycemic effect of different dose of Polygonati Rhizoma, i.e., “Ousei”, was investigated in both normal and streptozotocin-induced diabetic mice. The methanol extract of Polygonati Rhizoma (OM) (800 mg/kg) reduced the blood glucose of normal mice from 202 ± 7 to 144 ± 13 mg/100 ml 4 h after intraperitoneal administration (p < 0.01), and also lowered significantly the blood glucose of streptozotocin-induced diabetic mice from 589 ± 34 to 396 ± 15 mg/100 ml under similar conditions (p < 0.001). However, the hypoglycemic effects were not accompanied by any alteration in the serum insulin in these mice. OM also suppressed epinephrine-induced hyperglycemia in mice. These results support, therefore, the use of Polygonati Rhizoma in patients with diabetes and confirm its role as a traditional medicine. In addition, one of the active components of OM was identified as a spiristanol glycoside (PO-2).

Keywords hypoglycemic activity; Polygonati Rhizoma; oriental traditional medicine; streptozotocin; epinephrine; spiristanol glycoside

“Ousei” is the Oriental medicinal name given to Polygonati Rhizoma, rhizomes of Polygonatum falcatum A. Gray and the same genus plants. The medicine has been used traditionally for the treatment of patients with diabetes (polyuria and polydipsia). However, no experimental evidence has been produced of the hypoglycemic action of these materials. Isolation of some glycosides (PO-2, -3, -7, PF-1) from Polygonati Rhizoma has previously been reported without any pharmacological investigation of these compounds. The purpose of this study, therefore, was to examine the hypoglycemic effect and to isolated the active component from Polygonati Rhizoma.

Materials and Methods

Materials Plant materials used consisted of rhizomes of Polygonatum falcatum A. Gray collected in the medicinal plants garden of Kobe Women's College of Pharmacy (Japan), and also “Ousei” (Polygonati Rhizoma) obtained in a market in China by Tochimoto Tenkaido Co., Ltd. (Osaka, Japan). The data from this experiment were based on the latter material because, although both plant materials exhibited similar hypoglycemic activity in their corresponding active fractions, the former material was in too short supply to carry out many of the experiments concerning diabetes. Polygonati Rhizoma was extracted with methanol on a heating bath. The methanol extracts (OM) were lyophilized and stored at 4°C until just before use.

Animals Adult male ddY mice weighing 22−25 g were used. The mice were housed in an air-conditioned room at 24±1°C with a 12 h light and 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. Animals were divided into two groups. One group 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 the injection of STZ, the blood glucose levels of all the mice were determined. Mice with a blood glucose level above 300 mg/100 ml were considered to be diabetic and were used in this study. The hypoglycemic effect of OM was compared with that of tolbutamide and insulin in the normal and STZ-induced diabetic mice, respectively.

Oral Glucose Tolerance Test After overnight (18 h) fasting, mice were given the methanol extract of OM intraperitoneally and, 4 h later, glucose (2 g/kg body weight) solution was administered orally. Blood samples were collected before the administration of the glucose and at 0.5, 1 and 2 h later. Blood samples for serum insulin determination were also taken at 0.5 h after th administration of glucose.

Epinephrine-Induced Hyperglycemic Mice The adult ddY mice were given the extract intraperitoneally and, 3 h later, epinephrine (0.6 mg/kg body weight) solution was administered intraperitoneally. Blood and liver samples were collected 1 h after the administration of epinephrine.

Active Component The active component in OM was isolated by conventional methods for the preparation of glycosides, as summarised in Chart 1. OM was partitioned between n-butanol and water. The n-butanol layer was evaporated to dryness, adsorbed on silica gel and chromatographed on a silica-gel column, using chloroform-methanol-water (10:2:0.1) initially and subsequently changing the ratio (7:3:0.5). The crude substance was purified by silica-gel preparative TLC with n-hexane-AcOEt (2:3) as the development system. The compound gave colorless needles and a positive anisulfate reaction. The 13C-NMR spectral data of this compound were in accord with those published for PO-2 (Fig. 1). Moreover, this compound was characterized by performing a mixed melting point and comparing the Rf on TLC with that of an authentic sample (PO-2). By this means, the chemical structure was identified to be a spiristanol glycoside, PO-2. As PO-2 was assumed to be the active component in the methanol extract, by means of bioassy-guided fractionation, the compound was given to streptozotocin-induced diabetic mice.

Polyonatati Rhizoma (4 kg)
extracted with MeOH
MeOH extracts (183 g)
partitioned between n-BuOH and water
n-BuOH layer
defatted with n-hexane
aq. layer

Polyonatati Rhizoma (4 kg)
extracted with MeOH
MeOH extracts (183 g)
partitioned between n-BuOH and water
n-BuOH layer
defatted with n-hexane
aq. layer

PO-2 (802 mg)

Chart 1. Extraction and Separation of Polygonati Rhizoma

Fig. 1. Structure of PO-2

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Determination of Blood Glucose and Insulin Blood glucose levels in both normal and diabetic animals were determined by the glucose oxidase method\(^3\) and serum insulin was measured by a double-antibody radioimmunoassay.\(^4\) All the data were expressed as means ± 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.

Measurement of Liver Glycogen To the liver (1 g), 30% KOH solution (2.0 ml) was added and the mixture was boiled for 20 min. Ice-cold 95% ethanol (4.0 ml) was added to the mixture 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 means of the anthrone-H\(_2\)SO\(_4\) method.\(^5\)

Results

Effects of OM and Tolbutamide on Blood Glucose in Normal Mice The mean blood glucose levels in mice at various time intervals after intraperitoneal administration of OM are shown in Fig. 2. These levels were compared with the values in control mice administered saline alone and also in animals receiving 50 mg/kg body weight of tolbutamide, a known sulfonylurea hypoglycemic agent (Fig. 2). The hypoglycemic effect of OM was dose-dependent. OM (200, 400 mg/kg) had no effect on blood glucose, while OM (800 mg/kg) lowered blood glucose significantly from basal values of 202 ± 7 mg/100 ml to 144 ± 13 mg/100 ml (p < 0.01), 4h after the administration. OM (1600 mg/kg) also lowered blood glucose level at 4h (p < 0.01) and 7h (p < 0.05). The serum insulin levels in OM (800 mg/kg)-treated mice, however, did not change even at 4h (data not shown). At 10h, the blood glucose was unchanged. Tolbutamide-treated mice showed lower blood glucose levels over the period 2 to 7h after the administration.

Effects of OM and Insulin on Blood Glucose in Diabetic Mice The hypoglycemic effects of OM and insulin on the blood glucose of streptozotocin-induced diabetic mice are shown in Fig. 3. No differences in blood glucose were observed between the levels at the time points 2, 4, 7 and 10h after administration when compared with the basal values (at 0h) in control mice. However, OM (400, 800, 1600 mg/kg)-treated mice showed a significant decrease in blood glucose at 4h when compared with the basal values (OM 400: p < 0.05, OM 800, 1600: p < 0.001). The hypoglycemic effect of OM in STZ mice was also dose-dependently. Insulin-treated mice (5U/kg body weight) exhibited a significant decrease in blood glucose at 2h when compared with basal values (p < 0.001).

Oral Glucose Tolerance Test The glucose tolerance of ddY mice after oral glucose loading is shown in Table 1. OM-treated animals (800 mg/kg body weight, intraperitoneally) did not show a decrease in blood glucose when compared with controls. The serum insulin at 0.5h was 63 μU/ml in OM-treated mice, being similar to the value

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Before</th>
<th>30</th>
<th>60</th>
<th>120 (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>92 ± 6</td>
<td>249 ± 5</td>
<td>206 ± 31</td>
<td>130 ± 11</td>
</tr>
<tr>
<td>OM</td>
<td>800</td>
<td>78 ± 4</td>
<td>216 ± 21</td>
<td>191 ± 10</td>
<td>118 ± 5</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E. from 5 mice.

Table I. Effect of OM on Oral Glucose Tolerance Test

![Fig. 4. Effect of OM on Blood Glucose of Epinephrine-Induced Hyperglycemic Mice](image)

Each value represents the mean ± S.E. from 7 mice. Significantly different from OM(−), a p < 0.001.

![Fig. 3. Effects of OM and Insulin on Blood Glucose in Streptozotocin-Induced Diabetic Mice](image)

Each point indicates the mean ± S.E. from 5–6 mice. Significantly different from the baseline value, a p < 0.05, b p < 0.01, c p < 0.001. — — , control; — — , OM 200 mg/kg; — — , OM 400 mg/kg; — — , OM 800 mg/kg; — — , OM 1600 mg/kg; — — , insulin 5U/kg.

![Fig. 2. Effects of OM and Tolbutamide on Blood Glucose in Normal Mice](image)

Each point indicates the mean ± S.E. from 5–6 mice. Significantly different from the baseline value, a p < 0.05, b p < 0.01, c p < 0.001. — — , control; — — , OM 200 mg/kg; — — , OM 400 mg/kg; — — , OM 800 mg/kg; — — , OM 1600 mg/kg; — — , tolbutamide 50 mg/kg.
in controls.

Effect of OM in Epinephrine-Induced Hyperglycemic Mice The effect of intraperitoneally injected OM on epinephrine-induced hyperglycemia is shown in Fig. 4. OM-treated animals showed lowered blood glucose levels when compared with the untreated group (p < 0.001).

Effect of OM on Glycogen Content in Normal and Epinephrine-Induced Hyperglycemic Mice The glycogen levels in normal and epinephrine-induced hyperglycemic mice are shown in Fig. 5. In normal mice, the OM (800 mg/kg)-treated group exhibited a slightly increased glycogen content. In epinephrine-induced hyperglycemic mice, the OM (800 mg/kg)-treated animals exhibited increased glycogen levels when compared with the untreated group (p < 0.01).

Effect of Spirostanol Glycoside in Streptozotocin-Induced Diabetic Mice Spirostanol glycoside, PO-2 (Fig. 1, 50 mg/kg) which is the active component of OM, lowered blood glucose levels 4h after administration in STZ-induced diabetic mice (p < 0.01, Table II). But, the experimental specific activity (PO-2 per OM = 6.2% a change in blood glucose level) of PO-2 to OM did not give a reasonable ratio corresponding to the PO-2 content (0.43%).

Discussion
The present study clearly shows that the methanolic extract of Polygonati Rhizome (OM) produces consistent hypoglycemic effects in normal mice. The hypoglycemic effect was observed without any changes in serum insulin. In addition, we examined the therapeutic effects of OM on hyperglycemia in streptozotocin-induced diabetes, one of the animal models of insulin-dependent diabetes mellitus (IDDM). After the treatment of mice with OM, the resulting hypoglycemia was observed without any change in serum insulin. In terms of the hypoglycemic effect, the blood glucose in normal mice was lower than that in STZ-induced diabetic mice. Many Chinese medicines raise insulin sensitivity (for example Ginseng Radix*5). OM also had no effect on blood glucose levels in fasting mice. From these findings, it would appear that OM is used in diabetic therapy (IDDM).

In addition, OM decreased blood glucose and increased liver glycogen in epinephrine-induced hypoglycemic mice. We have already shown that OM decreases hepatic glucose production in isolated perfused rat liver (T. Miura et al., unpublished data). From these findings, it seems likely that OM may exhibit its hypoglycemic effects by decreasing the hepatic glucose output in both normal and epinephrine-induced hyperglycemic mice. It is possible to increase the glucose uptake in liver cell, because OM increases the glycogen content in normal mice and markedly lowers blood glucose levels in streptozotocin-induced diabetic mice. Moreover, we have isolated an active component associated with the hypoglycemic effect and have succeeded in identifying it as the spirostanol glycoside, PO-2. The yield of PO-2 from OM was 0.43%. However, the experimental specific activity (PO-2 per OM = 6.2% a change in blood glucose levels) of PO-2 to OM did not give reasonable ratio corresponding to the PO-2 content (0.43%). It is likely that the hypoglycemic effect of OM could be due to other compounds in OM as well as PO-2. In general, steroids (such as glucocorticoids) have been known to induce hyperglycemia, but this active component PO-2 seems to be an exceptional. It is, therefore, interesting that this steroidal glycoside, like spirostanol glycoside, exhibits a hypoglycemic effect in normal and diabetic mice, although the intracellular mechanisms in mouse hepatocytes are still unclear. It does not seem that OM inhibits the glyco-neogenesis of steroidal hormones (glucocorticoids), because OM did not decrease the blood glucose in fasting mice and had no effect on glyconeogenesis in isolated rat liver cells (data not shown). Further studies are needed to clarify this issue. The above experimental results suggest that the hypoglycemic effect of PO-2*4 supports the traditional medical use of Polygonati Rhizoma.

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References