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Studies on the Mechanism of the Hypoglycemic Activity of Ginsenoside-Rb_2 in Streptozotocin-Diabetic Rats

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The effect of ginsenoside-Rb_2 purified from ginseng was examined in streptozotocin-induced diabetic rats. The rats of the ginsenoside-Rb_2-treated group showed a significant decrease of blood glucose level. A moderate (but statistically insignificant) increase in the hepatic glycogen content was observed. Furthermore, the ginsenoside-Rb_2-treated group showed a significant rise of glucokinase activity in the liver, while there was a significant decrease in the activity of glucose-6-phosphatase. These results suggest that the changes of glucokinase and glucose-6-phosphatase levels probably represent one of the mechanisms of the hypoglycemic action produced by ginsenoside-Rb_2.

Keywords——ginsenoside-Rb_2; hypoglycemic activity; glycogen; glucokinase; glucose-6-phosphatase

From ancient times, the roots of *Panax ginseng* C. A. MEYER have been used as a therapy for the wasting syndrome, rather than as a specific therapeutic agent, in various diseases including diabetes mellitus, atherosclerosis, hypertension, and so forth. Studies on the hypoglycemic action of ginseng have been done by Saito,[1] Petkov,[2] Lei *et al.*,[3] and Kimura *et al.*[4−6] In particular, Petkov suggested that ginseng might have not only a synergistic action with insulin but also its own hypoglycemic activity.[2] On the other hand, we showed previously that a semi-purified saponin (fraction 4, 5) stimulates various metabolic reactions involved in lipid and sugar metabolism in normal rats.[7−11] Additional experimental results indicated that most of the biochemical actions of semi-purified saponin might be due to ginsenoside-Rb_2.[12,13] Administration of ginsenoside-Rb_2 to streptozotocin-induced diabetic rats reduced the level of blood glucose, producing an improvement of hyperglycemia.[14] These experiments prompted us to study the mechanism of hypoglycemic action of ginsenoside-Rb_2 in diabetic rats. The present paper deals with the effect of ginsenoside-Rb_2 on the activities in the liver of glucose-6-phosphatase (EC 3.1.3.9) and glucokinase (EC 2.7.1.2), enzymes which play an important role in the maintenance of blood glucose.[15] This report also describes the liver glycogen change produced by ginsenoside-Rb_2 treatment.

Materials and Methods

Animals——Male rats of the JCL: Wistar strain (Hokuriku Labour, Ltd., Toyama, Japan), initially weighing 90—100 g, were maintained in an air-conditioned room with lighting from 6 a.m. to 6 p.m. The room temperature (about 25 °C) and humidity (about 60%) were controlled automatically. A laboratory pellet chow (obtained from CLEA Japan Inc., Tokyo; protein 24.0%, lipid 3.5%, carbohydrate 60.5%) and water were given freely.

Streptozotocin-Induced Diabetic Rats[16]——Streptozotocin (65 mg/kg body weight) dissolved in 10 mM citrate

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buffer (pH 4.5) was injected intraperitoneally. Several days after the injection, blood glucose level was determined and the rats with a glucose level of 200 mg/dl or more were used.

Saponin——Ginsenoside-Rb_2 was isolated and purified from the extract of roots of *Panax ginseng* C. A. MEYER according to the procedure of Shibata and co-workers.[17] This preparation was found to be pure by various chemical and physicochemical analyses.

Treatment with Ginsenoside-Rb_2——Ginsenoside-Rb_2 (10 mg/rat/d) in saline was administered intraperitoneally to rats every day, while control rats were treated with an equal volume of saline. At 12 h after the last treatment, rats were sacrificed by means of a blow on the head and exsanguinated. Blood was collected in a conical centrifuge tube for the determination of glucose. The liver was removed quickly, cooled on ice, and weighed rapidly. A portion of the liver was homogenized in a Potter–Elvehjem type glass homogenizer with a Teflon pestle.

Chemicals——Adensosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADP) were obtained from P-L Biochemicals, Inc., U.S.A. Glucose-6-phosphate was obtained from Sigma Chemical Co., U.S.A. Glucose-6-phosphate dehydrogenase was purchased from Oriental Yeast Co., Tokyo, Japan. All other reagents were of the highest grade commercially available.

Statistics——The significance of differences between the control and ginsenoside-treated groups was tested by means of Student's *t*-test.

Determination of Glucose in the Serum——Glucose was determined by using a commercial reagent ("Glucose B-Test Wako" obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan) based on the glucose-oxidase method.[18]

Determination of Glycogen in the Liver——A portion of the liver was digested with 3 ml of 1 N NaOH in a boiling water bath for 60 min, and glycogen was precipitated by the addition of 2 ml of EtOH and purified according to the method of Roe and Dailey.[19] Liver glycogen was determined by the anthrone–H_2SO_4 method, with glucose as the standard.[20]

Determination of Enzyme Activities in the Liver——a) Glucose-6-Phosphatase: Liver was homogenized with 9 volumes of 0.25 M sucrose. The crude homogenate was centrifuged at $11000 \times g$ for 30 min and the precipitate was discarded. The supernatant fluid was further centrifuged at $105000 \times g$ for 60 min and the supernatant fluid was discarded. The solid precipitate (microsomes) was suspended in ice-cold sucrose–EDTA solution and stored at -20 °C until required. The activity of glucose-6-phosphatase was assayed according to the method of Baginski *et al.* with a slight modification.[21] The assay mixture contained 0.25 M sucrose–1 mM EDTA (pH 7.0), 0.1 M glucose-6-phosphate, and 0.1 M cacodylate buffer (pH 6.5) in a total volume of 0.3 ml. The reaction was started by the addition of a suitably diluted enzyme solution to the assay mixture. After incubation at 37 °C for 5 min, the reaction was terminated by the addition of 2 ml of 2% ascorbic acid–10% TCA solution. The precipitate formed was removed by centrifugation after the mixture had been left to stand in an ice-bath. A 0.5 ml aliquot was pipetted off and the liberated inorganic phosphate in the supernatant was determined by using a commercial reagent ("Phosphor B-Test Wako" obtained from Wako Pure Chemical Industries, Ltd.) based on the molybdenum blue method.[22]

b) Glucokinase: Homogenate of each liver was prepared in 2 volumes of homogenizing medium (0.15 M KCl containing 0.004 M $MgSO_4$, 0.004 M EDTA, and 0.004 M N-acetylcysteine, pH 7.0). This crude homogenate was centrifuged at $105000 \times g$ for 60 min. The supernatant fraction was used for the enzyme assay of glucokinase by the method of Walker and Parry.[23]

Results

Table I shows the blood glucose level of the ginsenoside-Rb_2-treated and control groups in streptozotocin-induced diabetic rats. The rats of the ginsenoside-Rb_2-treated group showed a significant decrease of blood glucose level; as shown in Table I, the blood glucose level was about 30% less at the 6th day in the ginsenoside-Rb_2-treated group as compared with the control group. These results were more effective than those of a single intraperitoneal administration, reported previously.[14] In contrast, administration of ginsenoside-Rb_2 had a lesser effect on the content of hepatic glycogen. As shown in Table I, an increase (27% compared to the control) in the glycogen content was observed on the 6th day after the repeated administration of ginsenoside-Rb_2 but was not statistically significant. The effect of ginsenoside-Rb_2 on the hepatic enzyme activities is shown in Table II. In the control group, glucose-6-phosphatase activity was increased about 2.4-fold in the liver of diabetic rats as compared with that of normal rats (16.7 *vs.* 40.4 μmol/min/mg protein). On treatment with the ginsenoside-Rb_2, this gluconeogenic enzyme activity in the liver decreased by about 31% as compared with that of the control animals. The data in Table II further indicate that the

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Table I. Effect of Ginsenoside-Rb_2 on Blood Glucose Level and Hepatic Glycogen Content

	Blood glucose (mg/dl)	Hepatic glycogen (mg/tissue)
Normal rat	117.7±7.8	179.8±18.1
Diabetic rat		
Control	730.8±33.1 (100)	45.4±5.8 (100)
Rb_2	511.6±49.4[a] (70)	57.8±8.2 (127)

Ginsenoside-Rb_2 was administered intraperitoneally to diabetic rats for 6 d, while the control group received an equal volume of saline. Values are means±S.E. of 6 rats. Figures in parentheses are percentages of the control value. *a*) Significantly different from the control value, $p<0.01$.

Table II. Effect of Ginsenoside-Rb_2 on Enzyme Activities in the Liver

	Glucose-6-phosphatase (μmol/min/mg protein)	Glucokinase (ΔOD/h/mg protein)
Normal rat	16.7±0.7	201.7±12.0
Diabetic rat		
Control	40.4±3.3 (100)	15.9±2.9 (100)
Rb_2	27.9±1.3[b] (69)	25.3±1.9[a] (159)

Ginsenoside-Rb_2 was administered intraperitoneally to diabetic rats for 6 d, while the control group received an equal volume of saline. Values are means±S.E. of 6 rats. Figures in parentheses are percentages of the control value. *a*) Significantly different from the control value, $p<0.05$, *b*) $p<0.01$.

ginsenoside-Rb_2-treated group of streptozotocin-induced diabetic rats showed a significant increase in glucokinase activity. This change was in reciprocal relation to that of glucose-6-phosphatase activity. However, compared to that of normal rats, the glucokinase activity of rats treated with ginsenoside-Rb_2 was markedly low.

Discussion

In this work we present evidence that ginsenoside-Rb_2 improves the hyperglycemia induced by streptozotocin. As for the mechanism of the hypoglycemic action, we postulate that ginsenoside-Rb_2 affects the glucose metabolism (gluconeogenesis, glycolysis, glycogenesis, and so forth).

As shown in Table II, the ginsenoside-Rb_2-treated group showed a significant rise of glucokinase activity in the liver, while there was a significant decrease in the activity of glucose-6-phosphatase. A moderate (but statistically insignificant) increase in the glycogen content was observed after the intraperitoneal administration of ginsenoside-Rb_2 (Table I). When glucose utilization is impaired in the liver of streptozotocin-induced diabetic rats, it may be considered that ginsenoside-Rb_2 produces its hypoglycemic activity by changing the levels of gluconeogenic and glycolytic enzymes and shifting the direction of the overall

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metabolic flow toward glucose degradation.

Following the experiments reported in the present paper, we observed that administration of ginsenoside-Rb_2 to diabetic rats stimulated the lipolytic activity of lipoprotein lipase, with a concomitant decrease in the level of triglyceride and very low density lipoprotein in the serum. Moreover, we observed that there was a significant accumulation of lipid in the adipose tissue (data not shown). These data, in conjunction with the preceding results on the improvement of hyperglycemia, suggest that ginsenoside-Rb_2 may play a role in facilitating the re-esterification of triglyceride fatty acid and glucose in the adipose tissue.

These actions are very similar to the metabolic alterations produced by insulin. Further work along this line is planned.

These results provide some evidence regarding the mechanism of hypoglycemic action of ginsenoside-Rb_2, and lend support to the view that ginsenoside-Rb_2 is useful for the therapy of hyperglycemia.

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