Original

Improving Effects of Ginsenoside-Rb_2 in Streptozotocin Diabetic Rats with Hyperglycemia and Hyperlipemia

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Effects of ginsenoside-Rb_2 on serum constituents were investigated in streptozotocin-induced diabetic rats. Administration of ginsenoside-Rb_2 reduced the level of blood glucose. However, the hypoglycemic effect appeared to be dependent on the level of blood glucose. The serum lipid-improving action was also observed in rats with hyperlipemia. Ginsenoside-Rb_2 treatment reduced serum triglyceride, non esterified fatty acid, and total cholesterol. In addition to the hypolipemic effect, ginsenoside-Rb_2 exhibited lowering action on the serum level of 3-hydroxybutyrate and acetoacetate in streptozotocin-induced diabetic rats, indicating an improvement of diabetic ketoacidosis.

Keywords·····ginsenoside-Rb_2; streptozotocin-induced diabetic rat; hypoglycemic action; serum lipid-lowering action

From ancient times, the roots of *Panax ginseng* C. A. MEYER has been used to the wasting syndrome, rather than a specific therapeutic agent of various diseases including diabetes mellitus in the Asian countries. From a biochemical standpoint, it displayed a variety of efficacies on the metabolism. 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] Furthermore, Yamamoto *et al.* reported that the oral administration of red ginseng powder was found to be effective in the improvement of hyperlipemia in diabetes mellitus.[7] On the other hand, we showed previously that a semi-purified saponin (fraction 4, 5) stimulates various metabolic reactions on lipid and sugar metabolism in normal rats.[8–12] Additional experimental results indicated that most of the biochemical action of semi-purified saponin might be due to ginsenoside-Rb_2.[13,14] These experiments prompted us to examine whether or not ginsenoside-Rb_2 is a useful agent on hyperglycemia and hyperlipemia due to diabetes. To investigate this problem, the present studies were undertaken to evaluate the effect of ginsenoside-Rb_2 in streptozotocin-induced diabetic rats.

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[15]·····Streptozotocin (65 mg/kg body weight) dissolved in 10 mM citrate buffer (pH 4.5) was injected intraperitoneally. Several days after the injection, blood

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glucose level was determined and the rats with a glucose level of 200 mg/dl or more were used as the diabetes.

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.[16] This preparation was found to be pure by various chemical and physicochemical analyses.

Treatment with Ginsenoside-Rb_2 ····· Ginsenoside-Rb_2 (10 mg/rat) made soluble in saline was administered intraperitoneally to the rats, while control rats were treated with an equal volume of saline. At the indicated time after intraperitoneal administration of ginsenoside, rats were sacrificed by means of a blow on the head and exsanguinated. Blood was collected and allowed to stand for several hours in a cold room at 4°C. Serum was separated by centrifugation (1000 x g, 10 min, 4°C).

Analyses·····Blood glucose, triglyceride, non-esterified fatty acid, and total cholesterol were determined by using a commercial reagent ("Glucose B-Test Wako" obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan; "TG-Five Kainos" obtained from Kainos Laboratories, Inc., Tokyo, Japan; "NEFA Kainos" obtained from Kainos Laboratories, Inc., Tokyo, Japan; "Cholesterol B-Test Wako" obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan). 3-Hydroxybutyrate was determined spectrophotometrically by measuring the increase of optical density at 340 nm, which was based on the oxidation of 3-hydroxybutyrate by 3-hydroxybutyrate dehydrogenase and NAD.[17] The determination of acetoacetate was based on the decrease in extinction at 340 nm due to the oxidation of NADH.[18] Lactate was determined by a spectrophotometric method, based on measurement of the increase of optical density at 340 nm.[19]

Statistics·····The significance of differences between the control and ginsenoside-Rb_2 treated groups was tested by means of Student's t-test.

Results

Table I shows the effect of ginsenoside-Rb_2 on the serum constituents after intraperitoneal administration. Ginsenoside-Rb_2 significantly reduced the blood glucose level by 19% at 12 hr as

Table I Effect of ginsenoside-Rb_2 on serum levels in rats treated with streptozotocin

Time after treatment (hr)	Glucose (mg/dl)	Triglyceride (mg/dl)	Non-esterified fatty acid (μEq/l)	T. cholesterol (mg/dl)
Control	704.8±31.6 (100)	451.0±58.5 (100)	834.5±64.4 (100)	153.3± 9.8 (100)
2	618.5±36.3 (88)	204.5±23.5[b] (45)	568.9±40.4[b] (68)	116.0± 6.8[a] (76)
4	648.0±27.4 (92)	234.1±24.0[b] (52)	579.5±47.6[b] (69)	112.4± 5.0[b] (73)
6	692.7±46.8 (98)	243.2±29.5[b] (54)	547.7±34.8[b] (66)	120.0± 4.5[a] (78)
8	668.5±32.1 (95)	299.2±24.1[a] (66)	534.3±34.2[b] (64)	113.4± 4.3[b] (74)
10	637.5±14.2 (90)	233.7±42.3[a] (52)	459.4±26.3[c] (55)	117.9± 9.7[a] (77)
12	573.8±18.9[b] (81)	182.1±22.9[b] (40)	361.8±50.6[c] (43)	115.7± 5.4[b] (75)
16	678.9± 5.8 (96)	346.5±34.5 (77)	869.9±55.5 (104)	142.6±11.4 (93)

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] $p<0.01$, [c] $p<0.001$.

compared with the control value, but had almost recovered to the control level 16 hr after the treatment. On the contrary, a striking change of triglyceride level in the serum was observed 2 hr after the intraperitoneal administration of ginsenoside-Rb_2; the mean values in the control and ginsenoside-Rb_2 treated groups were 451.0 and 204.5 mg/dl, respectively. A significant decrease was continued until 12 hr after the treatment. Similarly, administration of the ginsenoside-Rb_2 to rats resulted a significant decrease of the non-esterified fatty acid in the serum from 568.9 μEq/l at 2 hr to 361.8 μEq/l at 12 hr. The level of total cholesterol was also about 22-27% lower at 2-12 hr in the ginsenoside-Rb_2 treated group as compared with the control group. Thus, hyperglycemia and hyperlipemia induced with streptozotocin were much improved by ginsenoside-Rb_2 treatment.

Furthermore, experiments for the survey of ginsenoside-Rb_2 with hypoglycemic and hypolipemic activities were performed by using a rat having a blood glucose level of 140-770 mg/dl. The data in Fig. 1 demonstrate that among the rats used, blood glucose level was correlated with the triglyceride, non-esterified fatty acid, total cholesterol, 3-hydroxybutyrate, acetoacetate, and lactate levels in the rats without treatment of ginsenoside-Rb_2. Decrease in the blood glucose level was associated with declining triglyceride level ($r=0.80$). Similar relationships were observed with the non-esterified fatty acid, total cholesterol, 3-hydroxybutyrate, acetoacetate, and lactate. Especially, it is clear that there was a statistically significant relationship between the blood glucose and non-esterified fatty acid ($r=0.95$). Twelve hours after the intraperitoneal administration of 10 mg of ginsenoside-Rb_2, the

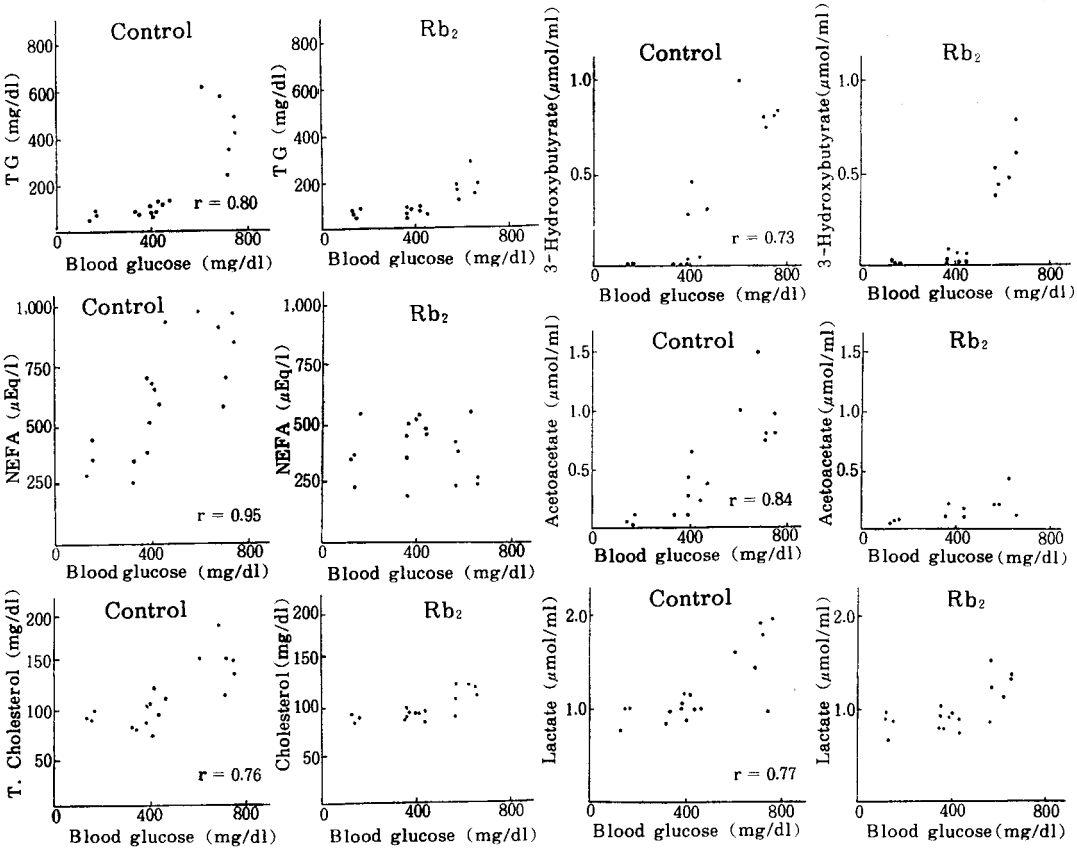

Fig. 1 Relation between blood glucose level and triglyceride, non-esterified fatty acid, total cholesterol, 3-hydroxybutyrate, acetoacetate, or lactate.

Table Ⅱ Effect of ginsenoside-Rb_2 on serum levels in rats treated with streptozotocin

	Glucose (mg/dl)	Triglyceride (mg/dl)	Non-esterified fatty acid (μEq/l)	T. cholesterol (mg/dl)	3-Hydroxy-butyrate (μmol/ml)	Acetoacetate (μmol/ml)	Lactate (μmol/ml)
Control	254.3±13.3 (100)	74.6± 5.3 (100)	370.3±38.8 (100)	94.4±4.0 (100)	0.03±0.01 (100)	0.08±0.02 (100)	0.95±0.06 (100)
Rb_2	224.5±19.9 (88)	73.8± 5.8 (99)	420.3±48.5 (114)	92.3±1.6 (98)	0.02±0.01 (67)	0.06±0.01 (75)	0.88±0.05 (93)
Control	425.4±16.7 (100)	104.2±10.8 (100)	663.0±72.3 (100)	101.1±7.3 (100)	0.23±0.08 (100)	0.37±0.08 (100)	1.02±0.04 (100)
Rb_2	387.0±16.2 (91)	69.4± 6.9[a] (67)	425.4±51.5[a] (64)	95.5±2.5 (94)	0.07±0.05 (30)	0.15±0.02[a] (41)	0.89±0.05 (87)
Control	690.7±31.6 (100)	405.9±58.5 (100)	817.8±64.4 (100)	153.3±9.8 (100)	1.10±0.25 (100)	0.98±0.11 (100)	1.63±0.15 (100)
Rb_2	573.8±18.9[b] (83)	182.1±22.9[b] (45)	361.8±50.6[c] (44)	115.7±5.4[b] (75)	0.53±0.26 (48)	0.19±0.06[c] (19)	1.25±0.19 (77)
Control	807.9±38.7 (100)	367.5±47.9 (100)	919.7±59.3 (100)	129.4±5.3 (100)	0.60±0.12 (100)	0.73±0.12 (100)	1.63±0.12 (100)
Rb_2	621.4±19.7[b] (77)	230.6±32.7 (63)	588.4±58.2[b] (64)	103.8±7.1[a] (80)	0.46±0.06 (77)	0.51±0.09 (70)	1.48±0.04 (91)

Values are means ± S.E. of 6 rats. Figures in parenthesis are percentages of the control values.
[a] Significantly different from the control value, $p<0.05$, [b] $p<0.01$, [c] $p<0.001$.

lowering action to hyperglycemia and hyperlipemia were confirmed. However, its effect of ginsenoside-Rb_2 appeared to be dependent on the level of blood glucose, triglyceride, non-esterified fatty acid, and total cholesterol.

For this, rats were divided into 4 groups according to the blood glucose level: 140-399 mg/dl (mean value 254.3 mg/dl), 400-469 mg/dl (mean value 425.4 mg/dl), 470-759 mg/dl (mean value 690.7 mg/dl), 760-870 mg/dl (mean value 807.9 mg/dl). The number of rats in each group was approximately equal. As a result, it was found that a significant decrease in the blood glucose level was observed in rats with the blood glucose level of 470-759 mg/dl (mean value 690.7 mg/dl) and 760-870 mg/dl (mean value 807.9 mg/dl) (Table Ⅱ). In addition, triglyceride in the serum was about 33-55% lower at a blood glucose level of 400 mg/dl or more in the ginsenoside-Rb_2 treated group as compared with the control group. In particular, treatment of rats with the ginsenoside-Rb_2 at a blood glucose level of 400-469 mg/dl (mean value 425.4 mg/dl) resulted in a normal or nearly normal serum level of triglyceride. A conspicuous decrease was also observed in the level of serum non-esterified fatty acid and acetoacetate at the blood glucose level of 400 mg/dl or more. Non-esterified fatty acid, which was 817.8 μEq/l, was decreased to 361.8 μEq/l in rats treated with ginsenoside-Rb_2. The level of acetoacetate decreased to 0.15 μmol/ml on the average at a blood glucose level of 400-469 mg/dl (mean value 425.4 mg/dl). On the other hand, a slight decrease was observed after the treatment in the level of lactate but there were no significant changes in the ginsenoside-Rb_2 treated and control groups.

Discussion

In this work we present evidences that ginsenoside-Rb_2 indicates an improvement of hyperglycemia induced with streptozotocin. As shown in Table Ⅰ, the level of blood glucose was significantly lower at 12 hr after administration as compared with the control value. However, the hypoglycemic effect of

ginsenoside-Rb_2 appeared to be dependent on the level of blood glucose. That is, a significant decrease in the blood glucose level was observed in rats with a blood glucose level of 470-759 mg/dl (mean value 690.7 mg/dl) and 760-870 mg/dl (mean value 807.9 mg/dl). Administration of ginsenoside-Rb_2 to rats caused no appreciable changes in rats with a blood glucose level of 140-399 mg/dl (mean value 254.3 mg/dl) and 400-469 mg/dl (mean value 425.4 mg/dl) (Table Ⅱ).

According to Saito,[1] *Panax ginseng* suppressed the hyperglycemia induced by epinephrine and high-carbohydrate diet. On the contrary, ginseng was also said to have little effect on the blood glucose level on the basis that any report could not define a significant change in blood glucose level by the administration of ginseng in normal rabbits. But in the other animal experiments, the administration of ginseng could normalize or suppress the hyperglycemic states induced by epinephrine, high-carbohydrate diet, or 2,8-dinitrophenol. In particular, Petkov notified that ginseng might have not only a synergistic action with insulin but also its own hypoglycemic activity.[2] Kimura *et al.* also confirmed the effect of ginseng on the lowering action of blood glucose in alloxan diabetic mice and anti-insulin diabetic mice.[4-6] In our observations, it is suggested that ginsenoside-Rb_2 purified from ginseng reduced the level of blood glucose in the streptozotocin-induced diabetic rats. Following the experiments reported in the present paper, the authors have been studying the hypoglycemic effect of ginsenoside-Rb_2 using a moderate diabetic rat. The results of our study have so far shown that ginsenoside-Rb_2 showed a tendency to gradually decrease of blood glucose level with increasing dosage.

Though the interpretation of the mechanism which exerts such effects remains to be elucidated, preliminary data from our laboratory indicate that ginsenoside-Rb_2 caused a significant increase of glucokinase activity in the liver of streptozotocin-diabetic rats, while there was no statistically significant difference in the activity of glucose-6-phosphatase. Considering these observations, it may be that hypoglycemic activity is brought about by means of changes in the levels of glycolytic and gluconeogenic enzymes, and shifting the direction of the overall metabolic flow toward glucose degradation. However, the authors observed that by the long-term administration of ginsenoside-Rb_2 to diabetic rats, the lipolytic activity of lipoprotein lipase was stimulated with a concomitant decrease in the level of triglyceride and VLDL-triglyceride in the serum. Moreover, we showed that there was a significant accumulation in the lipid content of adipose tissue.[20] As for the hypoglycemic action, it may be that ginsenoside-Rb_2 plays a possible role in facilitating the re-esterification of triglyceride fatty acid and glucose in the adipose tissue. The adipose tissue is assumed to be the most probable target organ of the ginsenoside-Rb_2.

These observations are very similar to alterations produced by insulin. However, the level of serum insulin was not significantly affected by ginsenoside-Rb_2.[20] Therefore, it was suggested that the observed phenomenon of ginsenoside-Rb_2 is not mediated by the insulin. From this point of view, further studies will be needed for the elucidation of the effect produced by ginsenoside-Rb_2.

In connection to this experiment, we also observed that there were significant changes in the concentration of serum lipids. In streptozotocin-induced diabetic rats, serum triglyceride, non-esterified fatty acid, and total cholesterol were elevated significantly (Fig. 1). Hyperlipemia due to diabetes was observed. On the contrary, ginsenoside-Rb_2 treatment reduced serum triglyceride, non-esterified fatty acid, and total cholesterol (Table Ⅰ, Ⅱ). Improving effects of ginsenoside-Rb_2 on hyperlipemia was observed in rats, but its effect was much greater in the triglyceride and non-esterified fatty acid levels than in the total cholesterol level. The mechanism of the effect has been studying in order to evaluate the possible therapeutic significance of the ginsenoside-Rb_2. The results so far obtained indicate an accumulation of lipid in adipose tissue as a result of its stimulating action on the lipolytic system. Further work along this line is planned.

Improving effects of ginseng saponin on hyperlipemia due to a high cholesterol diet feeding to rats were reported by Yamamoto *et al.*[21] The concentrations of total cholesterol and triglyceride in the serum were described to be decreased by the administration of ginseng saponin. Moreover, by the long-term administration of ginseng powder in hyperlipemic patients, they showed the evidences that serum HDL-cholesterol was elevated significantly and total cholesterol, atherogenic index, triglyceride, non-esterified fatty acid, and lipoperoxide levels were lowered significantly.[7] These data, in conjunction with our present experiments using ginsenoside-Rb_2, suggest that ginseng saponin including ginsenoside-Rb_2 is one of the excellent therapeutic agents for hyperlipemia due to a high cholesterol diet feeding and diabetes. Furthermore, it is of great interest to study the difference in biochemical effect of ginsenoside having different chemical structures.

In addition to the hypolipemic effect, ginsenoside-Rb_2 exhibited lowering action on the serum level of 3-hydroxybutyrate and acetoacetate in streptozotocin-induced diabetic rats, indicating an improvement of diabetic ketoacidosis (Table Ⅱ).

Finally, these results might give some evidence about the improving effect of ginsenoside-Rb_2 in streptozotocin diabetic rats with hyperglycemia and hyperlipemia, and might make way for clinical application of ginsenoside-Rb_2 to some condition of diabetes.

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