Original

和漢医薬学会誌 (Vol. 2 No. 2 1985)

Improving effects of diabetic symptoms by ginsenoside-Rb_2 in streptozotocin diabetic rats

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(Received June 11, 1985)

Abstract

Successive intraperitoneal administration of ginsenoside-Rb_2 was given to streptozotocin-induced diabetic rats (with blood glucose level of 430±30 mg/dl). Marked decrease in blood glucose level was obtained in ginsenoside-Rb_2 administered group. Their levels fell to about 160 mg/dl on the 6-9th day of the administration. On the other hand, body weight significantly increased in diabetic rats receiving ginsenoside-Rb_2, although they took less food than control. Ginsenoside-Rb_2 improved remarkably diabetic symptoms such as over-eating, polyuria, and glycosuria.

Key words ginsenoside-Rb_2, streptozotocin-induced diabetic rat, hypoglycemic activity, diabetic symptom

Abbreviation VLDL ; very low density lipoprotein

Introduction

In our previous paper we made it clear that among the various kinds of ginsenosides isolated from ginseng ginsenosides-Rb_2 exhibits a wide variety of biological activity in normal rats and accelerates glycogenolysis, glycolysis, and lipogenesis in the liver, and finally increases the content of triglyceride in the adipose tissue.[1,2] Moreover, we reported that a single intraperitoneal administration produced significant decrease in blood glucose level and marked improvement of hypertriglyceridemia in rats with streptozotocin-induced diabetes.[3-5] In addition, the mechanism of decrease in blood glucose level was revealed by giving successive intraperitoneal administration to rats.[6] Now, through the study on the effect of long-term administration of ginsenoside-Rb_2 we obtained the result suggesting that ginsenoside-Rb_2 may improve diabetic symptoms and reported it in this paper.

Materials and Methods

Animals : Male rats of the JCL : Wistar strain (CLEA Japan Inc., Toyama, Japan), initially weighing 110-120 g, were maintained in an air-conditioned room with lighting from 6 a.m. to 6 p.m. The room temperature (about 25℃) 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[7] : Streptozotocin (65 mg/kg body weight) dissolved in 10 mM citrate buffer (pH 4.5) was injected intraper-

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Journal of Medical and Pharmaceutical Society for WAKAN-YAKU 2, 372～376, 1985

itoneally. Several days after the injection, blood glucose level was determined and the rats with a glucose level of 430±30 mg/dl were used (normal rats 116±4 mg/dl).

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

Treatment with ginsenoside-Rb_2 : Ginsenoside-Rb_2 (10 mg/rat/day) in saline was administered intraperitoneally to rats every day at 9-10 a.m., while control rats were treated with an equal volume of saline.

Analytical methods : Blood 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.[9] Urinary glucose was determined by the method of Momose *et al.*[10]

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

Results

The changes in blood glucose levels were followed in rats with blood glucose level of about 430 mg/dl as shown in Fig. 1. When ginsenoside-Rb_2 was successively given at dose of 10 mg once a day, the blood glucose levels fell to 240 mg/dl after 3 days of administration and to about 160 mg/dl on the 6th day of administration. The level on the 9th day was similar to that on the 6th day. The administration was stopped after 9 days of administration and subsequently the changes in blood glucose level were followed. About 300

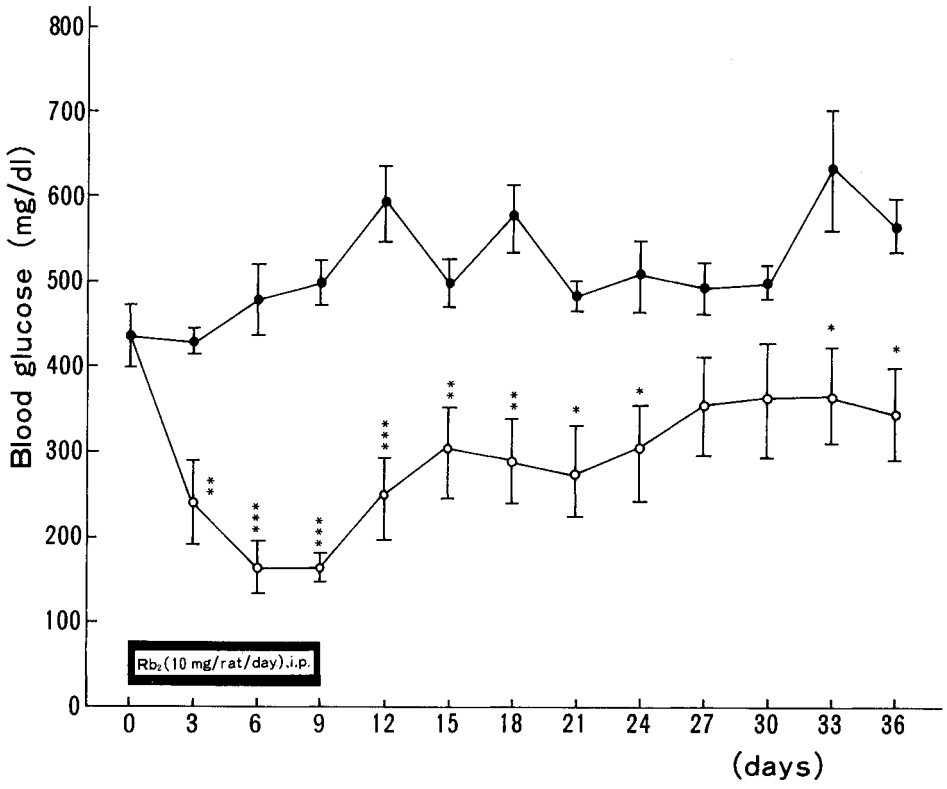

Fig. 1 Effect of ginsenoside-Rb_2 on blood glucose level.
Values are means±S.E. of 6 rats. *Significantly different from the control value, $p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$. ●—●, control group ; ○—○, ginsenoside-Rb_2 treated group.

mg/dl of blood glucose levels persisted up to the 36th day of the experiment. Therefore, low blood glucose level was maintained remarkably longer in the rats receiving ginsenoside Rb_2, compared with the control.

Next, during the period when the levels of blood glucose remained relatively constant after discontinuation of ginsenoside-Rb_2 administration, the measurement of urinary glucose and record of daily variation in blood glucose level were carried out by using a metabolic cage.

Figure 2 shows the relationship between intake of food and the change of body weight. Body weight increased significantly more in the group receiving ginsenoside-Rb_2. When they were kept in the metabolic cage, however, daily intake of food was smaller, in spite of larger weight gain.

Figure 3 shows intake of drinking water, urinary output, and the level of urinary glucose. Daily intake of drinking water was 90-110 ml in the control group, while it was remarkably low (20-40 ml) in ginsenoside-Rb_2 administered group. Similarly urinary output was remarkably low, near-normal level, in ginsenoside-Rb_2 administered group. The level of urinary glucose was 3-5 g/day in the control group, while it was extremely low (0.1-1.1 g/day) in ginsenoside-Rb_2 administered group. These results indicated that

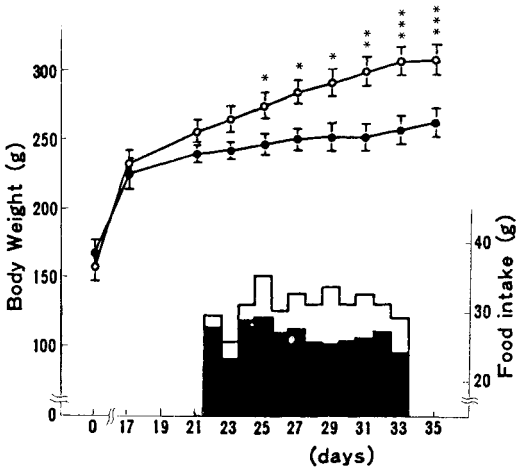

Fig. 2 Effect of ginsenoside-Rb_2 on the changes of body weight and food intake.
Values are means±S.E. of 6 rats. *Significantly different from the control value, $p<0.05$, **$p<0.01$, ***$p<0.001$. ●—● and □, control group ; ○—○and ■, ginsenoside-Rb_2 treated group.

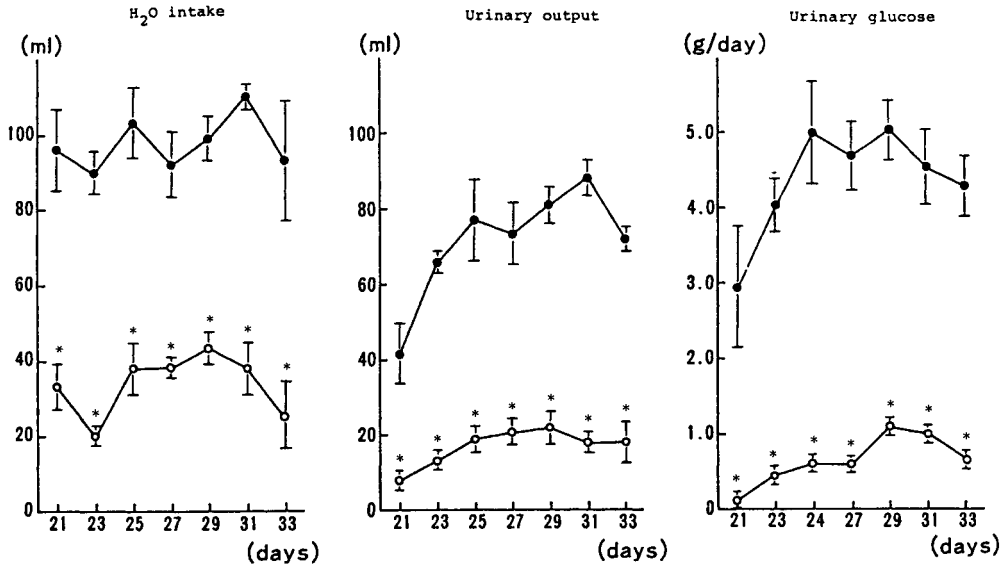

Fig. 3 Effect of ginsenoside-Rb_2 on H_2O intake, urinary output, and the level of urinary glucose.
Values are means±S.E. of 4 rats. *Significantly different from the control value, $p<0.001$.
●—●, control group ; ○—○, ginsenoside-Rb_2 treated group.

ginsenoside Rb$_2$ improves diabetic symptoms such as over-eating, over-drinking, polyuria, and excretion of sugar into urine.

In regard with daily variations in blood glucose level, ginsenoside-Rb$_2$ administered group showed lower blood glucose levels at any time, as shown in Fig. 4.

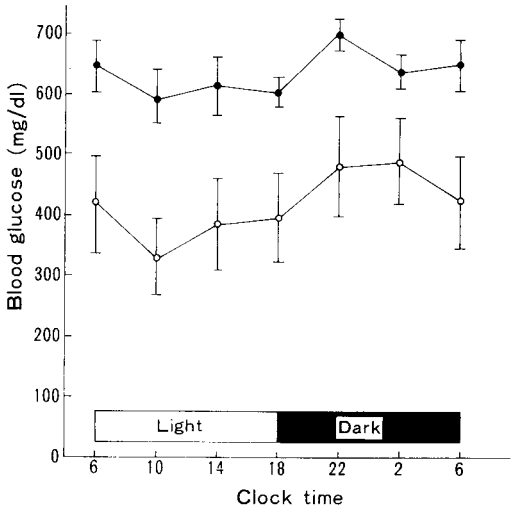

Fig. 4 Daily variation in blood glucose level.
This experiment was carried out at 35-35th day. Values are means±S.E. of 6 rats.
●—●, control group ; ○—○, ginsenoside-Rb$_2$ treated group.

Discussion

Dysbolism in diabetes is the abnormal metabolism of protein and lipid as well as carbohydrate. Because decrease in insulin action promotes release of sugar into the blood stream in the liver and decreases the uptake of glucose in muscle and adipose tissue, glucose level in the blood increases. When the blood glucose levels become higher than renal threshold, glucose synthesized in the liver leaks into the urine and is excreted before it is utilized for energy. In addition, high level of blood glucose produces the increase of plasma osmotic pressure and results in deficiency of water in the body due to osmotic diuresis. Finally, diabetic symptoms such as over-eating, over-drinking, polyuria, and dehydration may develop.[11]

In our experiment, rats with streptozotocin-induced diabetes showed abnormally high urinary output and urinary glucose level, delayed weight gain, over-eating, and over-drinking, compared with normal rats. However, diabetic rats receiving ginsenoside-Rb$_2$ showed significant increase in body weight, although they took less food than diabetic rats with no treatment. Moreover, their intake of drinking water, urinary output, and urinary glucose level were 1/4-1/5 the levels of control group. It was apparent that ginsenoside-Rb$_2$ has the continuous effectiveness for improvement of diabetic symptoms.

Acceleration of metabolism in the body by ginsenoside Rb$_2$ has been suggested to account for the mechanism of hypoglycemic effect of ginsenoside-Rb$_2$. In the experiment using rats with blood glucose level of more than 700 mg/dl, administration of ginsenoside-Rb$_2$ produced significant decrease in glucose-6-phosphatase activity, significant increase in glucokinase activity, and the tendency of increase in the level of glycogen in liver, as previously reported.[6] In addition, decrease of triglyceride and very low density lipoprotein (VLDL) levels in blood, increase of triglyceride content in adipose tissue, increase of lipoprotein lipase activity, and decrease of hormone-sensitive lipase activity were previously reported.[12] Therefore, these results suggest that ginsenoside-Rb$_2$ may increase the utilization of glucose and synthesize fatty acid in the liver by glycolytic system. This synthesized fatty acid may be transfered to adipose tissue via VLDL in the blood and stored as triglyceride.

On the other hand, diabetic rats receiving ginsenoside-Rb$_2$ showed no significant change in serum insulin level (data not shown). Based on this result, we would like to make further study on whether ginsenoside-Rb$_2$ may act as a kind of modifier which enhances action of insulin in the cell-side including insulin receptor or ginsenoside-Rb$_2$ may reduce the secretion of adrenaline, glucagon, or ACTH which acts as an antagonist against insulin.

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