Studies of nitrogen balance during ginsenoside-Rb_2 administration in streptozotocin-diabetic rats

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Abstract

The effect of ginsenoside-Rb_2 on nitrogen balance was investigated in rats with streptozotocin-induced diabetes. Ginsenoside-Rb_2 was found to suppress the total urinary excretion of nitrogen, increase nitrogen retention in the body and thereby improve nitrogen balance.

Key words ginsenoside-Rb_2, nitrogen balance, retentive nitrogen, urinary nitrogen, diabetic rat.

Introduction

It is known that the metabolic disorder induced by diabetes mellitus is reflected not only in carbohydrate and lipid metabolism but also in protein metabolism. In diabetes mellitus, the metabolic pattern in the body comes to resemble that seen in starvation, due to insulin deficiency, despite the fact that food is ingested. Therefore, the starvation-type regulation mechanism, which would normally act to maintain the homeostasis of the body, conversely gives rise to a vicious cycle.[1] We have so far investigated the effect of ginsenoside-Rb_2 on experimental diabetic rats, and have demonstrated that this compound partly restores these metabolic disturbances.[2-6] Consecutive intraperitoneal administration of ginsenoside-Rb_2 to diabetic rats resulted in an obvious, persistent decrease in blood glucose through increased channeling of metabolism into the glycolytic system, with evidence of improvement in diabetic symptoms such as body weight loss, polyphagia, polyposia, polyuria and glucosuria. A decrease in the level of urea in hepatic tissue, and an increase in the levels of ribosomal RNA and membrane-bound ribosomes suggested suppressed production of urea and improved protein biosynthesis in the hepatic tissue. When the state of protein biosynthesis was determined using labeled leucine, significantly increased incorporation into the serum protein was found in rats given ginsenoside-Rb_2. Also, determination of the distribution of [^{14}C]leucine in subcellular fractions prepared from the liver revealed a high activity in microsomal fraction. In the present study, the effect of ginsenoside-Rb_2 on nitrogen balance was studied in diabetic rats, as part of a research project on nitrogen metabolism.

Materials and Methods

Animals : Male rats of the Wistar strain, weighing about 250 g, were employed in this experiment. Diabetes was induced in the rats by intraperitoneal administration of streptozotocin (50 mg/kg body weight) dissolved in 10 mM citrate buffer (pH 4.5).[7] Two weeks after the injection, the blood glucose level was determined and rats with a glucose level of 410－495 mg/dl were used as diabetic rats. Before the study of nitrogen balance, all rats were housed in individual meta-

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bolic cages for 10 days and given an 18% casein diet composed of 18% casein, 57.9% α-cornstarch, 15% sucrose, 2% soybean oil, 4% salt mixture,[8] 1% vitamin mixture,[8] 2% cellulose powder and 0.1% choline chloride, and water *ad libitum*.

Saponin : Ginsenoside-Rb_2 was isolated and purified from a root extract of *Panax ginseng* C.A. MEYER produced in Kumsan, Korea. The structure of ginsenoside-Rb_2 was previously established by Sanada *et al.*[9] as 20S-protopanaxadiol-3-[O-β-D-glucopyranosyl (1→2)-β-D-glucopyranoside]-20-[O-α-L-arabinopyranosyl (1→6)-β-D-glucopyranoside].

Experimental procedure : The normal rats were fed an 18% casein diet. Diabetic rats were divided into 3 groups just before the study of nitrogen balance, *i.e.*, two given the same 18% casein diet and the other given a non-protein diet to estimate the endogenous nitrogen level. Just before urine collection, ginsenoside-Rb_2 (10 mg/rat/day) dissolved in saline was administered intraperitoneally to rats for 3 days, while control rats were treated with an equal volume of saline. Twenty-four-hour urine was collected for assay of daily nitrogen excretion throughout the balance study. Feces were also collected during the balance study and lyophilized. The food intake of each rat was recorded daily. The nitrogen content of the urine, feces and food samples was analyzed by a TN-5 or TN-10 total nitrogen analyzer (Mitsubishi Chemical Industries Inc.). The principle of this method is as follows. The sample boat is put into the reaction furnace preheated to a high temperature (800−900℃), using oxygen as a carrier gas. Nitrogen compounds in the sample are oxidized to NO. After removal of water with a demoisturizer, the NO is reacted with O_3 gas produced from the oxygen gas. Through this reaction process, light with a wavelength of 590−2500 nm is chemically generated. The light is amplified in a photomultiplier tube and analyzed by the data-processing section to obtain the level of nitrogen in the sample. The nitrogen level is calculated on the basis of the standard curve preliminarily obtained for a standard solution (ammonium sulfate). Absorbed nitrogen is calculated on the basis of ingested and fecal nitrogen using the equation shown below. Retained nitrogen is estimated from the absorbed nitrogen and urinary nitrogen.

Absorbed nitrogen = ingested nitrogen − (fecal nitrogen after protein diet − fecal nitrogen after non-protein diet)

Retained nitrogen = absorbed nitrogen − (urinary nitrogen after protein diet − urinary nitrogen after non-protein diet)

Statistics : Results were expressed as means ± S.E. of 6 rats. The significance of differences between the normal and diabetic rats (control or ginsenoside-Rb_2-treated group) was tested using Student's t test.

Results

Figure 1 shows the urinary excretion of nitrogen. In normal rats given the 18% casein diet, the level of urinary nitrogen was 220−260 mg/day during the experimental period, whereas in diabetic rats given the 18% casein diet, the corresponding level was 805−895 mg/day, showing markedly high values in comparison with normal rats. In contrast, in diabetic rats given ginsenoside-Rb_2, the levels of urinary nitrogen at 1−2 days and at 2−3 days were significantly decreased, being 17% and 16% lower, respectively, than those in the control group. In diabetic rats given the non-protein diet, the urinary nitrogen level was markedly decreased from the day after the beginning of the experiment, reaching a level corresponding to 15−20% of that in rats given the 18% casein diet. On the other hand, the level of ingested nitrogen throughout the entire experiment was maintained about 0.83 g/day in normal rats, whereas in the diabetic rats given the 18% casein diet, the value increased remarkably, becoming approximately double the normal value. However, there were no statistically significant differences between the control and ginsenoside-Rb_2-treated rats. The change in the fecal nitrogen level of each rat was essentially proportional

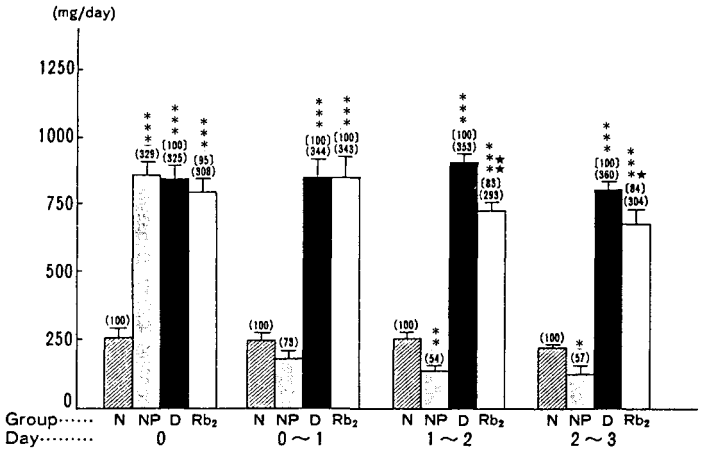

Fig. 1 Effect of ginsenoside-Rb_2 on urinary excretion of nitrogen.
N, normal rats ; NP, diabetic rats given non-protein diet ; D, diabetic rats given 18% casein diet (control group) ; Rb_2, diabetic rats given 18% casein diet (ginsenoside-Rb_2-treated group). Figures in parentheses are percentages of the normal or diabetic control value. ** ; Significantly different from the normal or diabetic control value, $p<0.05$, **** ; $p<0.01$, *** $p<0.001$.

Table I Absorbed and retained nitrogen at days 2–3 after ginsenoside-Rb_2 administration.

Group	Absorbed N (mg/day)	Retained N (mg/day)
Diabetic rat		
Control	1650±28 (100)	960±11 (100)
Rb_2	1745±78 (106)	1192±75 (124)*

Figures in parentheses are percentages of the control value. *Significantly different from the control value, $p<0.05$.

to the amount of ingested nitrogen.

Furthermore, the levels of nitrogen absorption and nitrogen retention in the body were obtained on days 2–3 following ginsenoside-Rb_2 administration, since the quantity of urinary nitrogen excreted by the rats given ginsenoside-Rb_2 was significantly decreased to 680 mg/day compared with 805 mg/day for diabetic rats given no ginsenoside-Rb_2, as shown in Fig. 1. On days 2–3, the level of ingested nitrogen was 1.79 g/day in the diabetic rats given ginsenoside-Rb_2 and 1.70 g/day in the untreated diabetic rats. The fecal nitrogen level was almost the same in the two groups (75.8 mg/day *vs.* 74.5 mg/day). Thus, the change in absorbed nitrogen calculated on the basis of ingested and fecal nitrogen exhibited a slight increase of about 6% in the rats administered ginsenoside-Rb_2 when compared with the control value, as shown in Table I, whereas administration of ginsenoside-Rb_2 significantly increased the level of retained nitrogen by 24% of the control value ; the retained nitrogen value was increased from 960 mg/day to 1192 mg/day ($p<0.05$).

Discussion

Nitrogen balance is widely used for judging the protein nutritional status in the body. Since nitrogen is almost completely excreted into the urine, the quantity of nitrogen excreted from the body is measured in terms of urinary nitrogen. The quantity of nitrogen retained in the body is calculated from the quantities of ingested nitrogen and excreted nitrogen. Nitrogen balance is thus obtained.[10]

As reported previously, rats with streptozotocin - induced diabetes show hypoproteinemia / hypoalbuminemia, increased blood urea nitrogen, increased urea and decreased RNA in hepatic tissue, together with decreased protein biosynthesis in serum and hepatic tissue, suggesting an incli-

nation of the nitrogen balance toward a negative value.[5,6] The results of the present study clearly showed that the level of urinary nitrogen excretion was 3.3 or 3.6 times higher in diabetic rats than in normal rats, indicating increased decomposition of somatic protein in diabetic rats. On the other hand, in comparison with normal rats, the ingestion of nitrogen was about double in diabetic rats, but the excretion of nitrogen was increased to a greater extent, resulting in inclination of the nitrogen balance toward the negative side. In contrast, when 10 mg of ginsenoside-Rb_2 was intraperitoneally administered to diabetic rats once a day for 3 days, the urinary excretion of nitrogen was significantly decreased by 17% (about 150 mg) at 1−2 days and by 16% (about 125 mg) at 2−3 days, with a tendency for the nitrogen balance to improve toward the positive side. Corroborating this, the nitrogen retention was significantly increased in the ginsenoside-Rb_2 administration group, although there were no significant differences in nitrogen absorption between the control and ginsenoside-Rb_2-administered groups. Ginsenoside-Rb_2-treated rats resulted in increased availability of ingested protein. Undersupply of glucose to muscle or other tissues due to insulin deficiency causes a decrease in the production of free energy, leading to energy supplementation through decomposition of protein, thus resulting in a decline in the nitrogen equilibrium to a negative value. It is considered that ginsenoside-Rb_2 converts this type of metabolic pattern to an anabolic one. Furthermore, there was facilitation of serum protein biosynthesis, a quantitative increase in hepatic ribosomes, and enhancement of protein biosynthesis in the hepatic microsomal fraction.[5,6] Therefore, it seems that the metabolic system in the body after ginsenoside-Rb_2 administration was oriented in the direction of body protein biosynthesis as well as improvement in nitrogen balance.

In modern Chinese medicine, ginseng is routinely used for the treatment of symptoms of vital force insufficiency among various conditions involving decreased physiological function. In view of its ability to supply "life energy," ginseng is given to patients with depression of spirits, hypofunction and lowered vital resistance in order to enhance systemic metabolic function.[11] The results obtained in the present study have thus provided scientific corroboration of some of the above experimental observations.

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和文抄録

ストレプトゾトシン誘発糖尿病ラットを用い，ginsenoside-Rb_2の窒素バランスに及ぼす影響を検討した。Ginsenoside-Rb_2の連日腹腔内投与により，尿中への総窒素排泄量の低下，体内に保留された窒素量の増加が認められ，窒素バランスの正への改善傾向が示唆された。

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