Biochemical Studies of Ginseng Saponin on RNA and Protein Biosynthesis in the Rat Liver

Hikokichi Oura

Department of Applied Biochemistry, Research for Wakan-Yaku, Toyama Medical and Pharmaceutical University 2630, Sugitani Toyama 930-01 Japan

Abstract

Previously, we reported that the intraperitoneal administration of ginseng crude saponin increased : (1) nuclear RNA polymerase activity, (2) nuclear RNA synthesis, (3) cytoplasmic RNA synthesis, (4) cytoplasmic heavy polyribosome content, (5) amino acid incorporation *in vitro* of microsome and polysome isolated rat liver, and (6) the incorporation rate of labeled amino acids into serum protein. In addition, a spectacular increase in the rough endoplasmic reticulum of hepatocyte administered crude saponin for four weeks orally was shown through electron microscopy. An increase in polysomal content in membrane-bound ribosome was shown through ultracentrifugation. Recently, successive intraperitoneal administration of ginsenosid-Rb_2 was given to streptozotocin (STZ) diabetic rats of hypoproteinemia. The blood urea nitrogen and hepatic urea concentration were decreased significantly. The total protein and albumin levels in the serum were increased in comparison to control values. In contrast, the ginsenoside-Rb_2 treated group of STZ diabetic rats showed a significant increase in liver RNA, total ribosome, and membrane-bound ribosomal contents. The administration of ginsenoside-Rb_2 increased the incorporation rate of labeled precursor into total serum protein. Additionally, ginsenoside-Rb_2 improved the nitrogen balance of diabetic rats. On the bases of these experimental results, ginseng saponin has a metabolic stimulatory or anabolic action on RNA and protein synthesis.

Introduction

Over the last twenty years or so, scientific research on ginseng has rapidly intensified, not only in Korea and Japan, but also worldwide. Many chemists and biologists, as well as clinical researchers are currently engaged in this field, and some of the results energing from these studies have proved to be highly valuable, contributing to the development of this old and traditional drug using modern methodology. Since 1965, we have been studying the biochemical action of ginseng extract and ginseng saponin.

Results

Effect of crude saponin on RNA and protein biosynthesis in normal rats[1-7]

In screening studies of several traditional tonic crude drugs, we found that an aqueous extract of ginseng produced a significant increase in the incorporation rate of labeled orotic acid into rapidly synthesized liver nuclear RNA after a single intraperitoneal injection. Table 1 shows that adminstration of an aqueous extract of ginseng radix, produced in Korea, increased the incorporation rate into liver nuclear RNA by 51% over the control value.

Table 1. Effects of Several Tonic Crude Drugs on the Incorporation of Labeled Orotic Acid into Rat Liver Nuclear RNA

Material	No. of rats	^{14}C-Orotic acid (cpm/mg RNA)	%
Control (saline)	6	58,250± 1,150	100
Radix Rehmanniae (China)	3	49,200	85
Fractus Lycii (China)	3	65,000	111
Rhizoma Cnidii (Japan)	3	57,600	99
Radix Ginseng (Korea)	3	87,900	151

Each crude drug extract was injected 0.5 ml per rat intraperitoneally, corresponding to 0.15 g of dried crude drug. ^{14}C-Orotic acid 2.5 μC were injected per rat respectively.

Chart 1. Purification of Acitive Component from Ginseng Radix

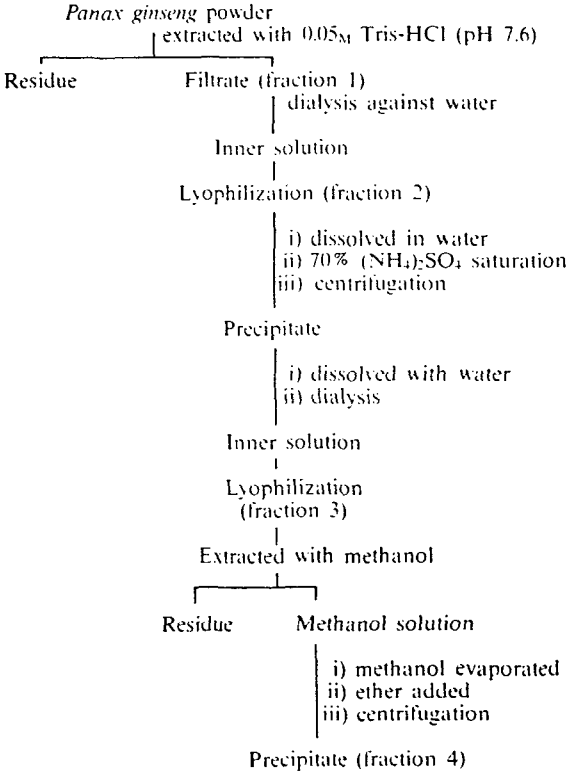


Chart 1 shows the method used for preparation of the biologically active fraction. Ginseng powder was extracted with Tris-HCl buffer for 48 hr in a cold room. A filtrate of the extract was centrifuged, the clear supernatant fluid obtained (fraction 1) was dialyzed against cold water, and then the internal solution was lyophilized (fraction 2).

Fraction 2 was 70%-saturated with ammonium sulfate, the resulting precipetate was dissolved in distilled water and dialyzed, and then the internal solution was lyophilized (fraction 3). Fraction 3 was extracted with methanol and then treated with cold ether to give a white or slightly yellowish precipitates as fraction 4.

The effects of the partially purified fractions 3 and 4 on the incorporation rates are shown in Table 2. Injections of 5 mg of these fractions produced rates of increase of 61% and 134%, respectively, in nuclear RNA orotic acid incorporation. The dose-response for fraction 4 is also shown.

Preliminary chemical examination of fractions 3 and 4 gave a positive Liebermann-Burchard reaction. By the colorimetric determination, the saponin content of fractions 3 and 4 was 35-40% and 90%, respectively. Therefore, a saponin-like substance was assumed to be the stimulating factor.

To obtain further information on the action of fraction 3, the time course of ^{14}C-orotic acid incorporation into nuclear RNA was determined. As presented in Fig. 1, it was evident that the greatest specific activity of nuclear RNA occurred at 4 hr after intraperitoneal injection of fraction 3, being increased by 50% over the control level. On the other hand, at 14 hr after administration of fraction 3, this response showed only a 20% increase and returned almost to the control rate at 24 hr after treatment.

The sedimentation profile of this rapidly labeled nuclear RNA examined using sucrose density gradient centrifugation is shown in Fig. 2. As reported by many workers with respect to normal nuclear RNA, we observed three absorbance peaks of RNA, which corresponded to ribosomal and transfer RNA. The stimulatory effect of fraction 4 on orotic acid incorporation differed for various RNA fractions with regard to the absorbance pattern, as shown in Fig. 2-B, and was greatest in the 10S region. This area roughly corresponds to mRNA.

DNA dependent RNA polymerase activity was determined after treatment *in vivo*. The stimulatory effect of fraction 4 was confirmed using graded doses, as shown in Table 3.

RNA polymerase activity was dependent on the amount of fraction 4 given. However, when fraction 3 or 4 was added to the reaction mixture for assay of polymerase activity *in vitro*, we were unable to observe any effect on RNA polymerase activity in liver slices or liver nuclei. Also, we failed to observe any increasing effect *in vitro* on the incorporation of orotic acid into RNA.

The maximum increase in DNA dependent RNA polymerase activity was 50% at 2 hr with a lag period of 30 min after treatment. The increase was still apparent at 3 and 8 hr after treatment, but was not siginficant after 16 and 24 hr (Fig. 3).

Figure 4 shows the effect of fraction 4 on the incorporation of orotic acid into polysomal RNA. From these data, it was evident that the greatest specific radioactivity of polysomal RNA was present at 6 hr after treatment relative to the control level. At 4 and 12 hr after treament, an increased level of specific radioactivity was still apparent. This response returned almost to the control level 26 hr after treatment.

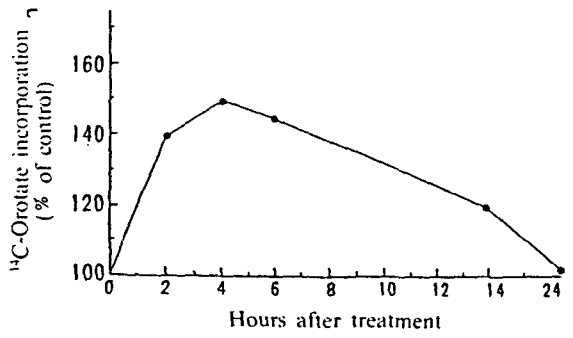
Figure 5 shows the increase in polysome content that was observed in liver cytoplasmic postmitochondrial

Table 2. Effect of Each Fraction isolated from Radix Ginseng on the Incorporation of ^{14}C-Orotic Acid into Rat Liver Nuclear RNA

Material	Does (mg)	No. of rats	^{14}C-Orotic acid (cpm/mg RNA)	%
Control (saline)	-	18	36,370± 900	100
Fraction 1	60[a]	6	54,500± 2,500	150
Fraction 1 (100, 10min)	60[a]	3	56,800	156
Fraction 2	5	3	55,500	153
Fraction 3	5	3	58,500	161
Fraction 4	0.01	3	40,600	112
Fraction 4	0.05	3	47,000	129
Fraction 4	1	3	54,700	151
Fraction 4	5	3	84,800	234
Actinomycin D	0.25	3	4,350	12

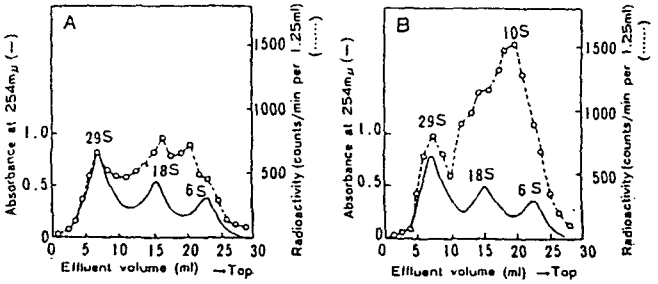
a) Corresponding to radix Ginseng powder. ^{14}C-Orotic acid (2.5 μCi/rat) was given intraperitoneally.

Fig. 1. Time Course of Effect of Fraction 3 Treatment on the Incorporation of ^{14}C-Orotic Acid into Liver Nuclear RNA



Fraction 3 (0.5 mg) was administered intraperitoneally to rats and ^{14}C-orotic acid (2.5μCi/rat) was injected intraperitoneally 20 min prior to killing the animals by decapitation. Three rat livers were pooled for each experimental or control group.

Fig. 2. Sucrose Density Gradident Analysis of Nuclear RNA



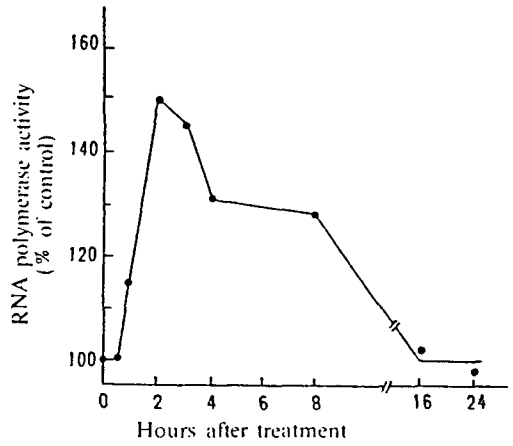
Fraction 4 (5 mg) was given to rats. At 4 hr after treatment ^{3}H-orotic acid (10μCi/rat) was injected intraperitoneally 20 min prior to killing the animals by decapitation. Each nuclear RNA of 10 absorbance units (at 260 mμ) were layered on a 0.5-1.0 M linear sucrose gradient.

Table 3. Effect of Graded Dose of Fraction 4 on RNA Polymerase Activity

Material	Dose (mg)	^{3}H-CMP incorporated cpm per mg protein	%
Control	-	2240	100
Fraction 4	0.1	2660	119
Fraction 4	0.5	2760	123
Fraction 4	1.0	2910	130
Fraction 4	5.0	3410	152

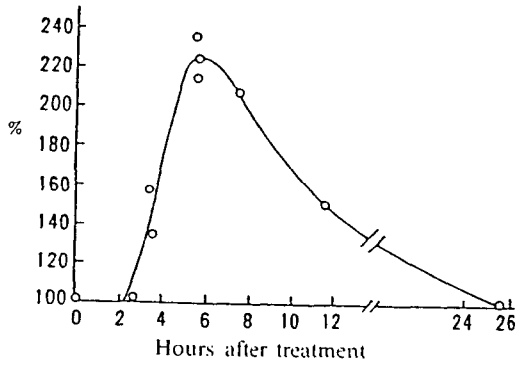
Polymerase activity was determined in the absence of ammonium sulfate.

Fig. 3. RNA Polymerase Activity of Liver Nuclei after Fraction 3 Treatment



Rats received a single dose of 5 mg of fraction 3 and were sacrified after varing time of the treatment. Nuclear enzyme was prepared from three pooled livers. Values shown in the figure were mean of two determinations for every enzyme preparation.

Fig. 4. Time Course of Effect of Fraction 4 Treatment on the Incorporation of ^{3}H-Orotic Acid into Cytoplasmic Polysomal RNA

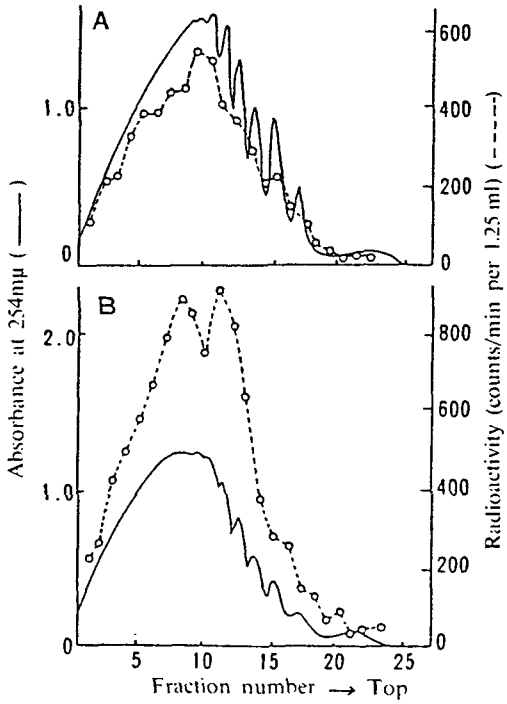


Fraction 4(5mg) was administered intraperitoneally rats at the designated time, ^{3}H-orotic acid (25μCi/rat) was injected intraperitoneally to animals, and the animals were killed 1.5 hr later by decapitation.

supernatant following fraction 4 treatment. The incorporation rate of ^{3}H-orotic acid into polysomal RNA and the population of polysomes were analyzed at 6 hr after treatment. Fraction 4 obviously enhanced the incorporation rate in terms of the total count present in the sucrose gradient. It was also found that the absorbance peaks of monomer, dimer, trimer and tetramer polysomes were markedly decreased by fraction 4 treatment in contrast to the region of heavy polysomes at 6 hr.

An attempt was then made to characterize the polysomal RNA induced by fraction 4 administration. Sedimentation analysis of rRNA and mRNA was carried out using a sucrose density gradient. The sedimentation diagram shows four radioactivity peaks, three of which coincide with the positions of the absorbance peaks of 29S and 18S ribosomal RNA and a mixture of 5S ribosomal RNA and 4S transfer RNA. The messenger RNA peak that contributes no significant UV absorption appears to be heterogeneous, as indicated by the wide 11S to 14S sedimentation range.

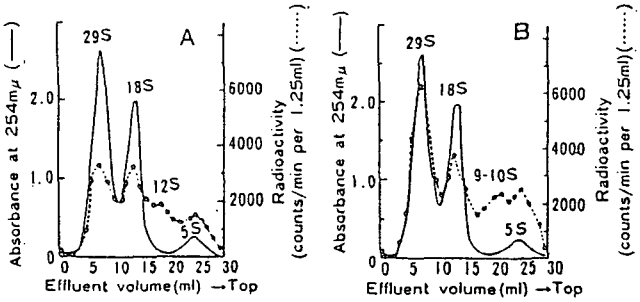
Fig. 5. Sucros Density Gradient Patterns of Purified Poly[illegible]



A) control (saline) input. 57 absorbance unit at 260mμ of polysome : a 0.3-1.0 M convex sucrose gradient centrifugation at 25000 rpm for 2.5 hr

B) At 4 hr after fraction 4 (5 mg/rat)-treatment, ^{3}H-orotic acid (25μCi/rat) was injected intraperitoneally and 1.5 hr later the animals were killed by decapitation. Input and centrifugation as in (A).

Fig. 6. Sucrose Density Gradient Analysis of Polysomal RNA from Control and Fraction 4-treated Rats



A) control : (saline) At 6 hr after intraperitoneal administration saline, ^{32}P-phosphate 3.0 mCi was injected per rat intraperitoneally at 2.5 hr prior to killing the animals, centrifugation at 24000 rpm for 40 hr.

B) At 6 hr after fraction 4 (5mg/rat) treatment, ^{32}P-phosphate (3.0mCi/rat) was injected intraperitoneally at 2.5 hr prior to killing the animals, centrifugation as in (A)

The sedimentation behavior of RNA induced by fraction 4 is shown in Fig. 6-B. The newly induced RNA is evenly distributed over the whole RNA spectrum. Particularly, 29S ribosomal RNA showed a significant increase in incorporation. In addition, we found that the radioactivity peak corresponding to messenger RNA shifted to the 9S-10S region from 11S to 14S in control liver (Fig. 6).

We determined the stimulatory activity of fraction 4 on microsomes and polysomes in an *in vitro* amino acid-incorporation system. It was found that the microsome

... 4. Stimulaton of ^{14}C-Leucine Incorporation into ...otein by Microsome and Polysome from Control and Fraction 4-treated Rats *in vitro*

Preparation	Treatment	^{14}C-Leucine (cpm/mg protein)	%
Microsome	Control(saline)	994	100
	Fraction 4	1,840	185
Polysome	Control(saline)	1,210	100
	Fraction 4	2,019	167

At 6 hr after administration of fraction 4(5 mg/rat), the animals were killed by decapitation.

Table 5. Effect of Fraction 3 on the Incorporation of ^{3}H-Leucine into Serum Protein

Time after fraction 3 treatment (hr)	No. of rats	^{3}H-Leucine (cpm/0.1 ml serum)	%
Control	5	555± 34	100
4	3	759± 45	137
8	3	811± 39	146
12	3	826± 40	149
16	3	644± 60	116
20	3	617± 26	111

At the desired time after fraction 3 (5 mg) administration, each rat received intraperitoneally 4 μCi of ^{3}H-leucine. One hr later, blood sample was taken by heart puncture.

Table 6. Effect of Fraction 3 on th Rate of Synthesis of Serum Protein, Albumin and γ-Globulin

	Reciprocal of dilution No. of serum	^{3}H-Leucine cpm Control	Experiment	%
cpm in 0.1 ml of serum		4,606	6,706	46
cpm in albumin-anti-albumin ppt	1.5/800	195	255	31
	2.0/800[a]	201	276	37
cpm in γ-globulin-anti-γ-globulin ppt	2.0/25	398	796	100
	2.5/25[a]	447	856	93

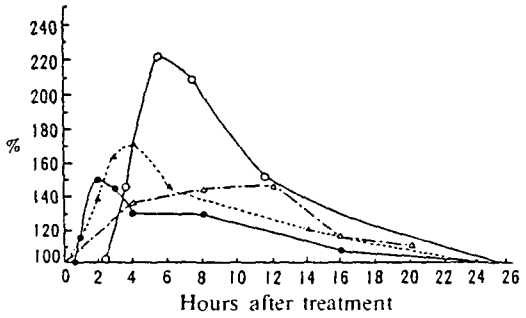
Rats were injected intraperitoneally with 5 mg of fraction 3. These rats received intraperitoneally 20μCi of ^{3}H-leucine 9 hr after fraction 3 administration. One hr later, blood samples were taken.[a] at equivalence zone.

and polysome fractions obtained from the liver of fraction 4-treated rats showed more active incoporation of labeled amino acid than the corresponding fractions obtained from normal liver (Table 4).

As seen in Table 5, a significant increase in the incorporation rate of labeled leucine into serum protein was observed 4 hr after intraperitoneal injection of 5 mg of fraction 3. It reached a maximum level about 8-12 hr after injection, representing a 46-49% increase. The rate of synthesis decreased to the control level 20 hr after treatment.

To characterize the serum protein formed at an enhanced rate by administration of fraction 3, immunochemical precipitation was employed. Anti-rat serum albumin and anti-γ-globulin were prepared from rabbit antisera and the proportion of antigen to antibody in the equivalence zone was determined. As seen in Table 6, albumin-anti-albumin precipitates showed a 37% increase, and a 93% increase in the rate of γ-globulin synthesis was observed at the equivalence zone following fraction 3 administration.

Fig. 7. Summary of Data on the Sequential Stimulations by Fraction 3 or 4.



●, liver nuclear RNA polymerase activity ; ▲, nuclear RNA synthesis ; ○, polysomal RNA synthesis ; ▲, serum protein synthesis.

Here we summarize the sequential stimulation of biochemical effects (Fig. 7). The first phenomenon observed was stimulation of the Mg-activated DNA-dependent RNA polymerase activity beginning 1 hr after administration, reaching a 50% increased level at 2 hr. The second phenomenon was an increase in the specific radioactivity of rapidly labeled nuclear RNA, attaining a maximum rate at 4 hr after treatment. A substantial rise in polysomal RNA synthesis in the cytoplasm took place about 6 hr after fraction 4 had been given, thus elevating the hepatic polysome content and protein synthesis activity. Finally, synthesis of serum protein was gradually stimulated, reaching a maximum level at about 8-12 hr after treatment. However, all these activities returned almost to the respective control levels about 24 hr after treatment.

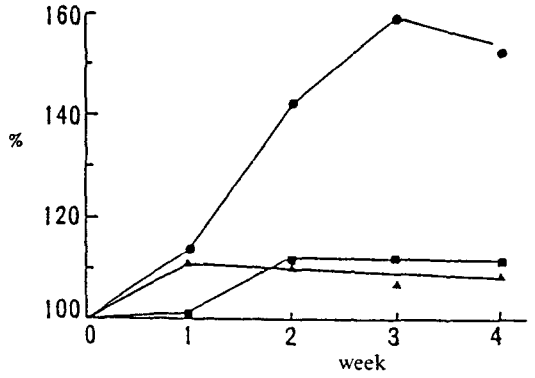
In this work, administration of fraction 3 or 4 enhanced the rate of synthesis of serum protein. On the basis of these experimental results, we suggest that the action of ginseng extract is metabolic stimulation of RNA and protein biosynthesis. This stimulatory factor, which for convenience we have termed "protein synthesis-stimulating factor", prostisol, has an active effect on RNA and protein synthesis when administered *in vivo*.

By long-term administration, an attempt was made to clarify whether fraction 3 caused an increase in the endoplasmic reticulum and ribosome contents of rat liver. Experimental rats given fraction 3 orally for 4 weeks were examined. An increase in rough endoplasmic reticulum in hepatocytes, relative to controls, was found by electron microscopy. The amounts of RNA in liver homogenate, postmitochondrial supernatant and polysomes were assayed at 1, 2, 3 and 4 weeks after fraction 3 administration.

Figure 8 shows the effect of fraction 3 pretreatment on the amount of RNA and polysomes in rat liver. It was found that the RNA was increased by about 10% over the control level in both the homogenate and postmitocondiral supernatant for 1 to 4 weeks. However, it was observed that the amount of polysomal RNA isolated from postmitochondrial supernatant increased significantly to 59% and 52% over the level in control rats at 3 and 4 weeks, respectively.

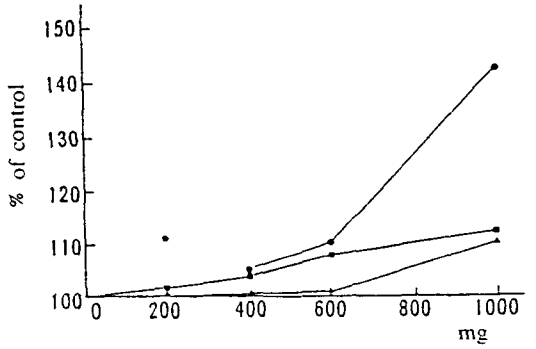
The dose-response effect of fraction 3 was studied at 2 weeks after oral administration. As shown in Fig. 9, the RNA content in both postmitochondrial supernatant and the polysome fraction was directly dependent on the

Fig. 8. Effect of Fraction 3 on RNA Content in Rat Liver



Fraction 3 was administered to rats orally at 1000 mg/kg body weight of rat/day for 1 to 4 weeks. ■, homogenate; ▲, postmitochondrial supernatant; ●, polysomal RNA

Fig. 9. Effect of Dose-reponse of Fraction 3 on RNA Content in Rat Liver



Fraction 3 was administered to rats orally at 200 mg/kg to 1000 mg/kg body weight of rat/day for 2 weeks. ■, homogenate; ▲, post-mitochondrial supernatant; ●, polysomal RNA

Table 7. Effect of Fraction 3 on Ribosome Content in Rat Liver

		Total ribosome	membrane-bound ribosome	Free ribosome
Control	mg RNA/g liver	3.18	2.20	0.98
	ratio	100	69	31
Fraction 3	mg RNA/g liver	4.00	3.09	0.92
	ratio	100	77	23
% of control		126	140	94

The amounts of ribosome were assayed at 4 weeks after oral administration of fraction 3 (1000 mg/kg body weight of rat/day).

amount of fraction 3 given. However, the RNA content of liver homogenate was not increased until 600 mg fraction 3/kg body weight had been administered. These experimental results would seem to suggest that RNA synthesis in the liver was stimulated by fraction 3 in the nucleus and that the increased RNA was transferred to the cytoplasm without accumulation.

Ribosomes in rat liver cells are present either free or attached to the endoplasmic reticulum membrance. A quantitative study of the distribution of ribosomes between these two states was carried out by centrifugation with and without DOC treatment.

Table 7 shows that the amount of total ribosomes was increased by 26% in fraction 3-treated groups as compared with the control rats. Also, membrane-bound ribosomes were increased 40% by fraction 3 for 4 weeks orally administration. On the other hand, no significant difference was observed in free ribosome content between the control and treated groups. These experimental results suggest that the increase in ribosomes caused by administration of fraction 3 is mainly due to an increase in membrane-bound ribosomes.

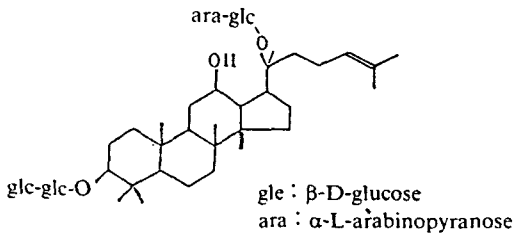
Effect of ginsenoside-Rb_2 on RNA and protein biosynthesis in streptozotocin diabetic rats.

In a previous paper,[8-14] we reported that among the various kinds of ginsenosides, Rb_2 showed a wide variety of biochemical activities, accelerating glycogenolysis, glycolysis and lipogenesis in the liver, and increasing the content of triglyceride in adipose tissue. Moreover, we reported that Rb_2 (Fig. 10) treatment of rats with streptozotocin-induced diabetes produced a significant decrease in the blood glucose level and a marked improvement of hyperlipemia.

STZ-induced diabetes is known to impair liver protein synthesis, and several mechanisms including decreased RNA polymerase activity, decreased total RNA, decreased specific mRNA and a decrease in polyribosomes have been proposed.[15] Also, this type of diabetic rat shows decreased incorporation of labeled amino acid into serum protein and reduced levels of several plasma proteins. From these experimental findings, we examined whether Rb_2 is an agent capable of improving the nitrogen metabolism of hypoproteinemic diabetic rats.

Ginsenosude-Rb_2 was successively given at a dose of 10 mg/rat/day for 6 days intraperitoneally. Figure 11 shows that the blood urea nitrogen (BUN) concentration was increased about 2.8-fold in diabetic rats as compared with normal rats. The Rb_2-treated group showed a significant decrease (43%) of urea nitrogen. Also, a remarkable increase in hepatic urea concentration was

Fig. 10. Ginsenoside-Rb_2

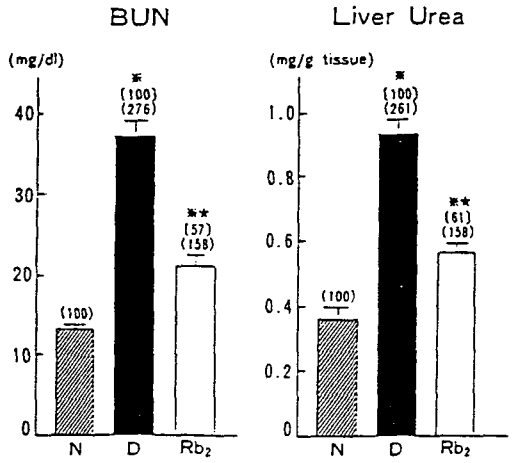


S. Sanada, N. Kondo, J. Shoji, and S. Shibata: Chem. Pharm. Bull., 22, 421 (1974)

observed in diabetic rats as compared with the normal group. However, the Rb_2-treated group showed a significant decrease (40%). These results show that nitrogen metabolism in the liver of diabetic rats can be improved by Rb_2 administration.

Figure 12 shows the serum free amino acid concentrations. Diabetic rats showed significantly decreased levels of Lys, Gly, Glu and Arg as compared with non-diabetic normal rats, while the concentrations of Pro, Leu, Val, Ileu and Cit were significantly increased.

Fig. 11. Effect of Ginsenoside-Rb_2 on Bun and Liver Urea



N. Normal ; D. Diabetes ; Rb_2. Diabetes (Rb_2 10 mg/rat/day i.p. for 6 days)
** $p<0.001$

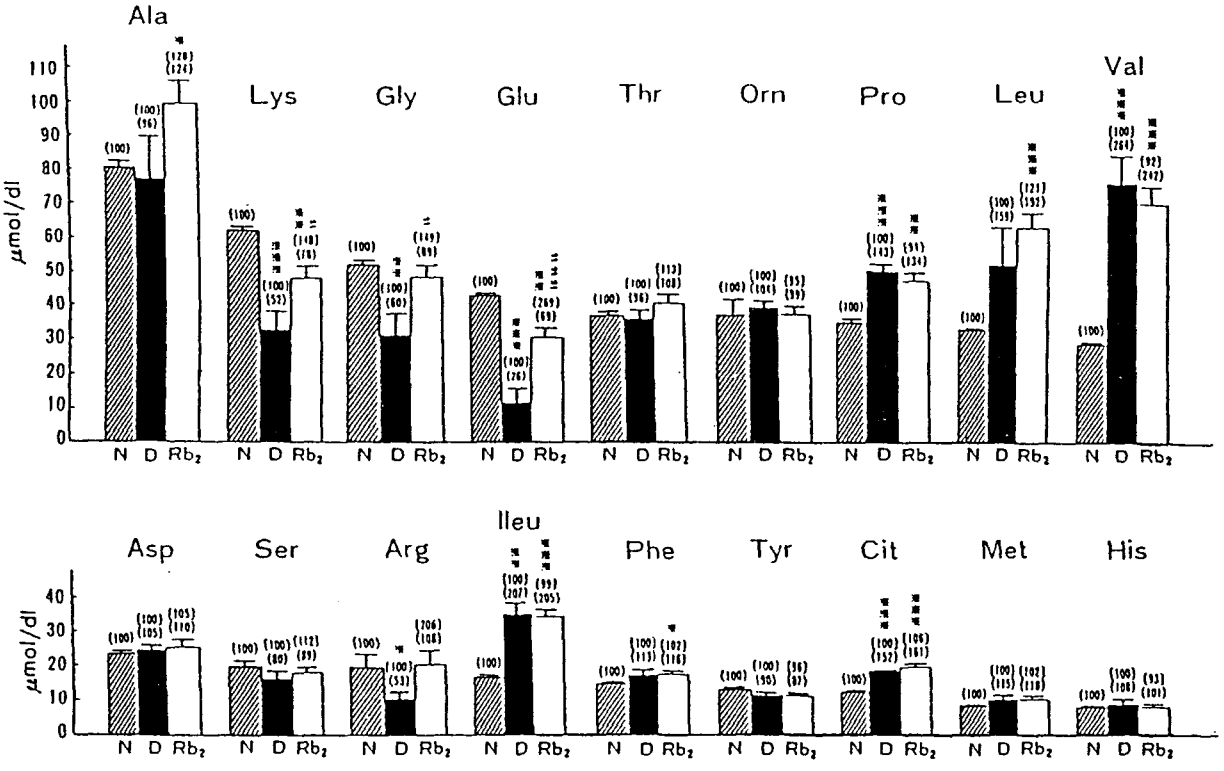
However, the Lys, Gly, Glu and Arg levels in the Rb_2-treated group of diabetic rats were significantly higher than those in the control group. No change was seen in the levels of Pro, Leu, Val Ileu and Cit.

The effect of ginsenoside-Rb_2 in the liver was shown in Fig. 13. The concentrations of Glu, Ala, Thr, Hia, Phe, Tyr, Met and Arg were significantly decreased in diabetic rats as compared with the non-diabetic rats. However, the concentrations of Ser, Leu, Orn, Asp, Val, Ileu and Cit were increased. Ginsenoside-Rb_2 had a normalizing effect on the hepatic concentrations of Glu, Thr, Phe and Tyr.

Figure 14 shows the effect of Rb_2 on the protein, RNA and DNA contents of rat liver. In diabetic rats, liver RNA content was decreased by 30% as compared with normal rats, while the Rb_2-treated group showed a significant increase in RNA content. However, protein and DNA in the liver showed no changes.

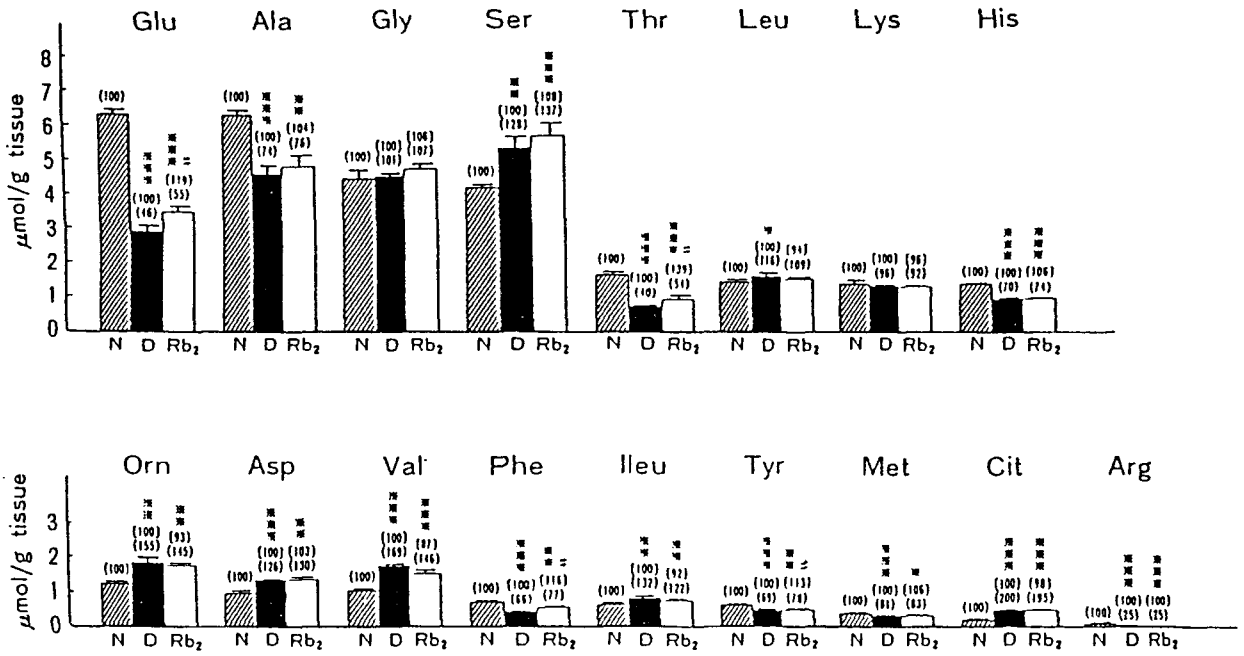
Figure 15 shows the ribosome content of the liver. In diabetic rats, total and free ribosomal RNAs were decreased significantly as compared with normal rats. Membrane-bound ribosomes also showed a moderate decrease, but this was not significant. The amounts of total ribosomes showed a 22% increase following Rb_2 treatment as compared with control diabetic animals. Similarly, administration of Rb_2 resulted in a significant increase in membrane-bound ribosomes, showing a normalizing effect. The amount of membrane-bound ribosomes was increased by 28% with Rb_2 administration. However, no significant difference in free ribosome content was observed between the diabetic group and Rb_2-treated rats.

Fig. 12. Effect of Ginsenoside-Rb_2 on Free Amino Acid in the Serum



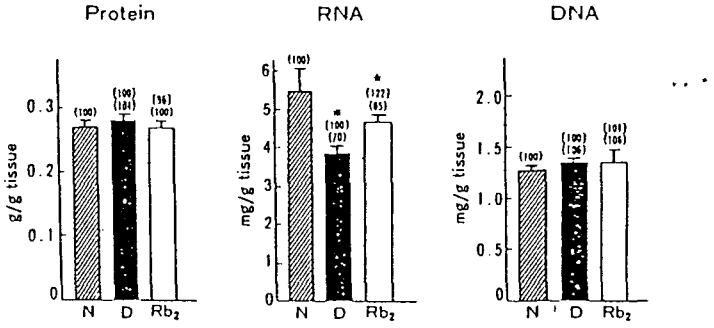
N. Normal : D : Diabetes. Rb_2 Diabetes (Rb_2 10 mg/rat/day. i.p. for 6 days). * $p<0.05$. ** $p<0.001$.

Fig. 13. Effect of Ginsenoside-Rb_2 on Free Amino Acid in the Liver



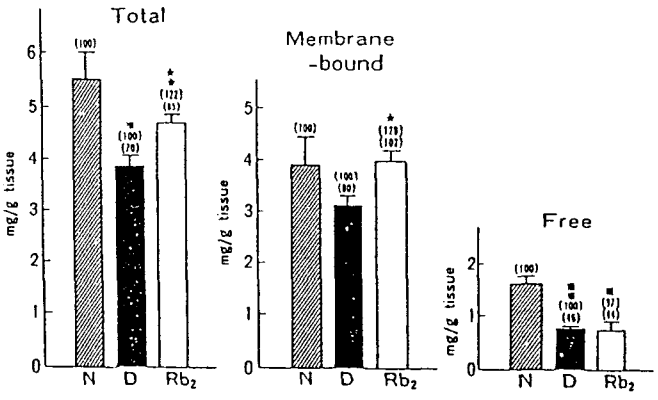
N ; Normal, D ; Diabetes, Rb_2 ; Diabetes (Rb_2 10 mg/rat/day i.p. for 6 days). * $p<0.05$

Fig. 14. Effect of Ginsenoside-Rb_2 on Protein, RNA and DNA Contents in the Liver



N ; Normal, D ; Diabetes, Rb_2 ; Diabetes (Rb_2 10 mg/rat/day i.p. for 6 days) *$p<0.01$, **$p<0.001$: * $p<0.05$, ** $p<0.01$

Fig. 15. Effect of Ginsenoside-Rb_2 on Ribosomal RNA Content in the Liver



N ; Normal, D ; Diabetes, Rb_2 ; Diabetes (Rb_2 10 mg/rat/day i.p. for 6 days) *$p<0.01$, **$p<0.001$, *$p<0.05$, ** $p<0.01$

Table 8. Effect of Ginsenoside-Rb_2 on the Incorporation of ^{3}H-Orotic Acid into Hepatic Nuclear RNA after 6 Days of Administration

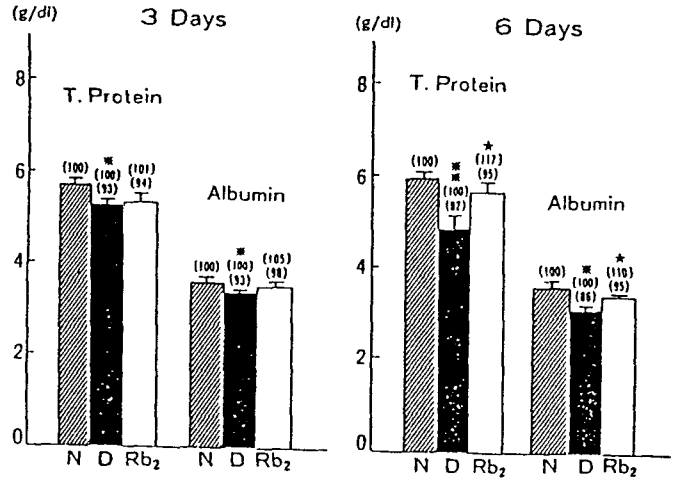
Group	Incorporated radioactivity (cpm/mg nuclear RNA)	
Non-diabetic rat	11925 (100)	
Diabetic rat		
Control	9649 (81)	(100)
Rb_2	16074 (135)	(167)

^{3}H-Orotic acid 25μCi/rat, i.p., labeling time 20 min.

In order to confirm this ribosomal RNA-increasing activity of Rb_2 treatment, we determined the incorporation of ^{3}H-orotic acid into liver nuclear RNA after 6 days of Rb_2 administration. In diabetic rats, incorproated specific radioactivity was decreased by 20% as compared with normal rats. The Rb_2-treated group showed an increase of 35% and 67% as compared with the normal and diabetic groups, respectively (Table 8). These experimental results support the notion that the ribosome content of the liver is increased by Rb_2 administration.

Consecutive intraperitoneal administration of Rb_2 resulted in a decrease in the level of urea in hepatic tissue, an increase in the levels of hepatic ribosomal RNA and membrane-bound ribosomes, and partial normalization of the level of free amino acids in the blood. These findings seemed to suggest facilitation of protein biosynthesis, and therefore the effects of Rb_2 on protein biosynthesis were determined by measuring the incorporation of labeled precursor.

Fig. 16. Effect of Ginsenoside-Rb_2 on Serum Constituents after 3 or 6 Days of Administration



N. Normal ; D. Diabetes ; Rb_2. Diabetes (Rb_2 10 mg/rat/day. i.p.)
. p<0.05. ** p<0.01

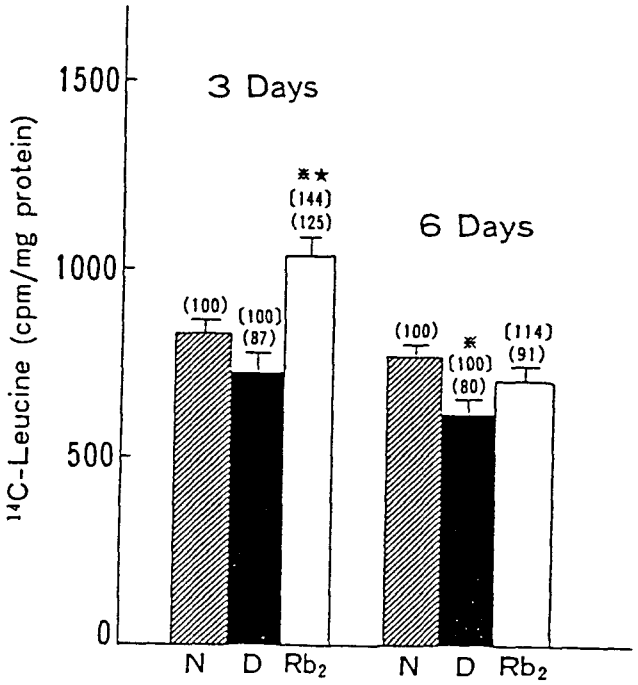
First. the serum protein and albumin levels were measured. The total protein was significantly decreased in diabetic rats as compered with the non-diabetic normal rats. The gruop treated with Rb_2 for 6 days showed a significant increase in total protein from 4.86 to 5.68 g/dl, a 17% change. However. after 3 days of Rb_2 adiministration. the total protein level showed no appreciable change. Similar changes were also observed in the albumin level (Fig. 16).

Figure 17 shows the effect of Rb_2 on the incorporation of ^{14}C-leucine into serum protein. A dose of 10 mg of Rb_2 was given intraperitoneally for 3 or 6 consecutive days. and the effects of the two periods of administration on protein biosynthesis were compared. After 3 days of Rb_2 administration. the incorporation rate into serum protein was significantly enhanced. At the 6th day in the Rb_2-treated group. the incorproation of radioactive precursor was also higher than that in the control group, but this variation was not statistically significant.

As shown in Fig. 18. in the next experiment. the distribition of radioactivity in hepatic subcellular fractions was examined. As significantly activated serum protein biosynthesis was observed in rats given Rb_2 for 3 days. this period was used for consecutive administration of Rb_2 treatment. increased incorporation of labeled amino acid was observed in the homogenate. mitochondrial and soluble fractions. A moderate increase was also found in the microsomal fraction. but this was not statistically significant. In the nuclear fraction. no change attributable to Rb_2 adminstration was seen.

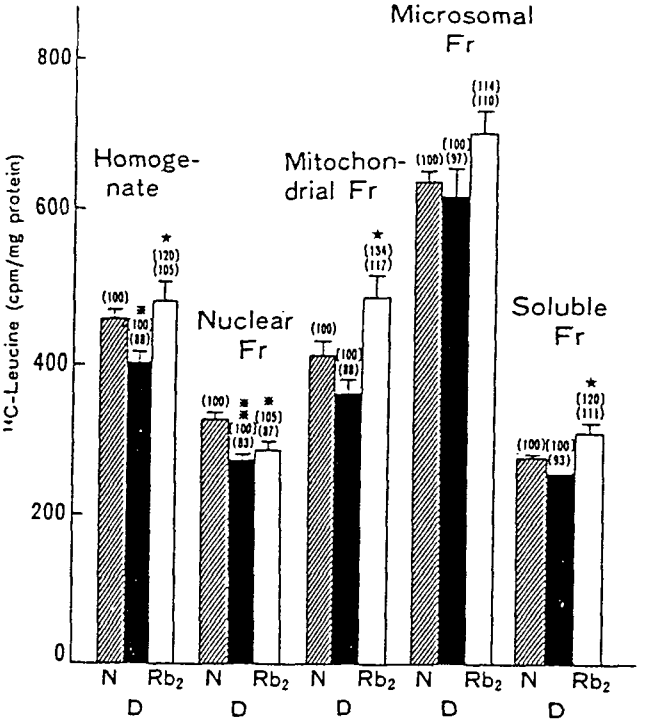
Figure 19 shows the urinary excretion of nitrogen. In normal rats given an 18 % casein diet. the level of urinary nitrogen was about 250 mg/day during the experimental period. whereas in diabetic rats given an 18% casein diet. the corresponding level was about 800 -1000 mg/day. showing markedly high values in comparison with normal rats. In contrast. in diabetic rats given ginsenoside-Rb_2. the levels of urinary nitrogen on days 1-2 and days 2-3 were significantly decreased. being 17% and 16% lower than those in the control

Fig. 17. Effect of Ginsenoside-Rb_2 on the Incorportation of ^{14}C-Leucine into Serum Protein



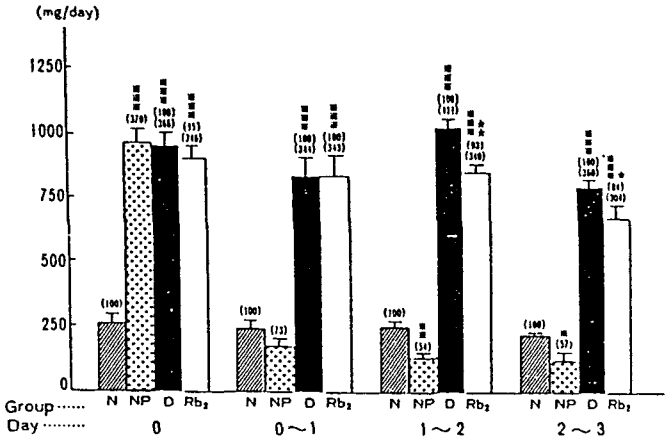
N. Normal ; D. Diabetes
Rb_2. Diabetes (Rb_2 10 mg/rat/day. i.p.)
^{14}C-Leucine 4μCi/rat. i.p. ; Labeling time 1 hr
** p<0.01

Fig. 18. Effect of Ginsenoside-Rb_2 on the Incorporation of ^{14}C-Leucine Protein of Subcellular Fraction in Liver



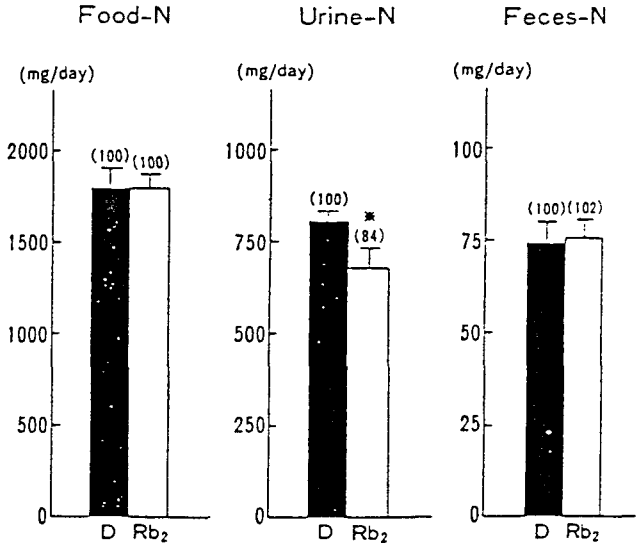
N: Normal ; D. Diabetes ; Rb_2. Diabetes
(Rb_2 10 mg/rat/day. i.p.. for 3 days)
^{14}C-Leucine 4μCi/rat. i.p.. labeling time 1 hr.** p<0.05. ** p<0.01

Fig. 19. Effect of Ginsenoside-Rb_2 on Urinary Excretion of Nitrogen



N, Normal ; NP, Non-Protein ; D, Diabetes
Rb_2, Diabetes (Rb_2 10 mg/rat/day, i.p.)
, $p<0.05$; **,** $p<0.01$; *** $p<0.001$

Fig. 20. Effect of Ginsenoside-Rb_2 on Nitogen Content in Food, Urine and Feces



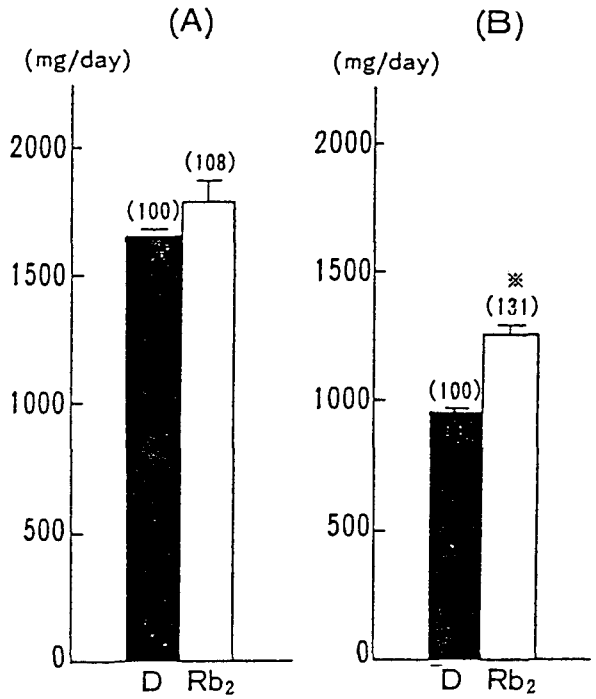
D, Diabetes ; Rb_2 Diabetes (Rb_2 10 mg/rat/day, i.p. for 3 days)
* $p<0.05$

group, respectively. In diabetic rats given a non-protein diet, the urinary nitrogen level was markedly decreased from the day after the beginning of the experiment. The level was 130-180 mg/day during the experimental period.

In this experiment, rats were maintained on the basis of a pair-feeding schedule. Thus, the amount of nitrogen ingested during the experimental preiod was the same between the control and ginsenoside-Rb_2-treated groups. Fecal nitrogen also showed equal levels. However, at 3 days of ginsenoside-Rb_2 administration, the amout of excreted nitrogen was significantly decreased in comparison with the control group (Fig. 20).

Absorptive and retentive nitrogen were calculated using the equation shown below.

Fig. 21. Absorptive N (A) and Retentive N (B)



D, Diabetes : Rb_2, Diabetes (Rb_2 10 mg/rat/day, i.p. for 3 days)
* $p<0.001$

Absorptive N = ingested N − (fecal N after protein diet − fecal N after non-protein diet)
Retentive N = absorptive N − (urinary N after protein diet − urinary N after non-protein diet)

The level of absorptive nitrogen was increased by 8%, although this was statistically insignificant. However, retentive nitrogen was significantly increased by 31% by ginsenoside-Rb_2 administration. These results show that ginsenoside-Rb_2 exerts an anabolic action by making the nitrogen balance more positive (Fig. 21)

Summary

The effect of ginseng saponin of RNA and protein biosynthesis was examined in normal or diabetic rats. The experimental results obtained were as follows :

1. Liver nuclear DNA dependent RNA polymerase activity is increased.
2. Liver nuclear RNA bisynthesis is increased.
3. Liver cytoplasmic ribosome content is increased.
4. Liver membrane-bound polysome content is increased.
5. Liver protein biosynthesis is increased.
6. Serum protein biosynthesis is increased.
7. Liver urea and blood urea nitrogen are decreased..
8. Urinary urea excretion is decresed..
9. Retentive nitrogen in the body is increased.
10. Nitrogen balance is improved.

References

1. H. Oura, S. Hiai, S. Nakashima and K. Tsukada, *Chem. Pharm. Bull.*, 19, 453 (1971).
2. H. Oura, S. Hiai and H. Seno, *Chem. Pharm. Bull.*, 19, 1598 (1971).

H. Oura, K. Tsukada and Y. Hirai, *Chem. Pharm. Bull.*, 19, 1656 (1971).
H. Oura, K. Tsukada and H. Nakagawa, *Chem. Pharm. Bull.*, 20, 219 (1972).
H. Oura, S. Nakashima, K. Tsukada and Y. Ohta, *Chem. Pharm. Bull.*, 20, 980 (1972).
H. Oura, S. Hiai, Y. Odaka and T. Yokozawa, *J. Biochem.*, 77, 1057 (1975).
H. Oura, S. Hiai, S. Nabetani, H. Nakagawa, Y. Kurata and N. Sasaki, *Planta Medica*, 28, 76 (1975).
T. Yokozawa, T. Kobayashi, H. Oura and Y. Kawashima, *Chem. Pharm. Bull.*, 32, 2766 (1984).
T. Yokozawa, T. Kobayashi, A. Kawai, H. Oura and Y. Kawashima, *Chem. Pharm. Bull.*, 32, 4490 (1984).
T. Yokozawa, T. Kobayashi, H. Oura and Y. Kawashima, *J. Med. Pharm. Soc. WKAKN-YAKU*, 1, 22 (1984).
T. Yokozawa, T. Kobayashi, H. Oura and Y. Kawashima, *Chem. Pharm. Bull.*, 33, 869 (1985).
T. Yokozawa, T. Kobayashi, H. Oura and Y. Kawashima, *Chem. Pharm. Bull.*, 33, 3893 (1985).
T. Yokozawa, M. Kiso, H. Oura, S. Yano and Y. Kawashima, *J. Med. Pharm. Soc. WKAKN-YAKU*, 2, 372 (1985).
T. Yokozawa, H. Oura and Y. Kawashima, *Chem. Pharm. Bull.*, 35, 4872 (1987).
L.S. Jefferson, W. S. L. Liao, D. E. Peavy, T. B. Miller, M. C. Appel and J. M. Taylor, *J. Biol. Chem.*, 258, 1369 (1983).

K. S. Lee : what is the effect of ginsenosides on the normal rat? Is ginsenoside effect related to diabetic or is it the general effect on metabolism on normal?

H. Oura : I can't understand. Rb_2 have hormone-like action. From the viewpoint of serum protein biosynthesis, we have studied using various pure ginsenoside. As a result, ginsenoside-Rd is more effective on the incorporation rate of ^{14}C-leucine into serum protein. Since ginsenoside-Rb_2 accelerate the various biochemical activities, one of the effect of this saponin may be related to the metabolism of the body. However, I would like to examine whether or not the effect of ginsenoside-Rb_2 is improved to histological damage of pancreas itself.

J. K. Park : I know that the pancreatic B-cell is damaged specifically by the streptozotocin. If the B-cell damage can not be overcome by the dosage of STZ in your experiment, it seems like that the blood glucose level will increase again. What do you think about it?

H. Oura : In this afternoon. Dr. Yokozawa will speak about sugar and lipid metabolism.

간에서의 RNA, 단백질 생합성에 미치는 인삼성분의 생화학적 연구

Hikokichi Oura

Department of Applied Biochemistry, Research for Wakan-Yaku, Toyama Medical and Pharmaceutical University 2630, Sugitani, Toyama 930-01 Japan

조사포닌을 복강내 투여하면 1) 핵내의 RNA polymerase의 활성도, 2) 핵내의 RNA합성, 3) 세포질의 RNA합성.4) 세포질내의 폴리리브좀 함량, 5) *in vitro* 상태에서 쥐간의 polysome과 microsome으로의 아미노산 유입율, 6) 방사능 표지된 아미노산의 혈청단백질로의 유입율이 증가하였음을 과거에 보고 한 바 있으며 또한 4주간 조사포닌을 투여한 쥐에서 적출한 간세포를 전자 현미경으로 조사한 결과, 조면 소포체가 상당히 증가하였으며 초원심분리기로서 막에 결합한 ribosome에서의 polysome함량의 증가를 확인하였다. 최근 streptozotocin으로 유도한 단백질 결핍성 당뇨병 쥐에 Rb_2를 계속적으로 주사한 결과 blood urea nitrogen과 간내의 urea 농도가 현저히 감소하였으며 혈청내의 총단백질과 알부민의 농도가 대조군의 수치에 비하여 증가한 반면 간내의 RNA와 총 ribosome, 막에 결합된 ribosome의 함량이 증가하였다. 또한 Rb_2투여로 혈청내의 총단백질로의 방사능 표지 전구물질의 유입량이 증가하였으며 당뇨쥐에서의 질소균형을 개선시켰다. 이러한 실험적 결과에 근거하여 인삼 사포닌은 대사를 촉진시키고 RNA, 단백질 합성 등을 촉진하는 것으로 생각된다.