METABOLIC ACTIONS OF GINSENG PRINCIPLES IN BONE MARROW AND TESTES

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Panax ginseng has been widely used in the oriental medicine since over 2,000 years. Chemical, pharmacological and biochemical investigation on ginseng, however, was rather recently begun. The extensive work has been made by Prof. Shibata and his colleagues about chemical structures of saponins and sapogenins in ginseng as Prof. Shibata mentioned before. As to biochemical actions of ginseng, Prof. Oura and his colleagues found that ginseng extract stimulated the RNA and protein synthesis in the rat liver, as Dr. Hiai mentioned yesterday.

We have been engaged in studies of the biochemical actions of ginseng principles since several years, especially on synthesis of nucleic acids, protein and lipid in bone marrow, testes, adipose tissue and cancer cells.

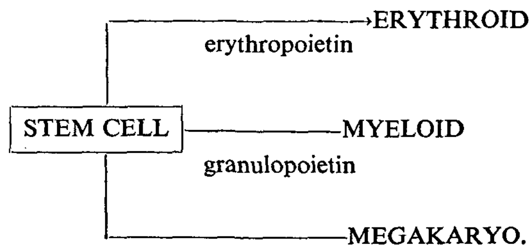
We assumed that the major active principles of ginseng might be saponins, the chemical structures of which had been already determined by Prof. Shibata and his colleagues.

In this symposium, I would like to address some data about biochemical actions of ginseng extract and ginsenosides on bone marrow and testes.

Biochemical actions on bone marrow

Bone marrow is known to be one of the most active organs in mitosis with DNA synthesis, producing red blood cells, white blood cells and platelets.

Hematopoiesis in bone marrow.



Erythropoiesis in bone marrow.

STEM CELL
| erythropoietin
PROERYTHROBLAST
|
ERYTHROBLAST (BASO.)
|
ERYTHROBLAST (POLYCHR.)
|
ERYTHROBLAST (NORMOCHR.)
|
RETICULOCYTE
↓
ERYTHROCYTE

In both erythroid and myeloid, it is said that cell division takes place 4 times during maturation process. DNA and RNA synthesis is most active in proerythroblasts and decreases as maturation proceeds. The red cells as well as white cells contain much protein and lipid, too. Synthesis of materials such as DNA, RNA, protein and lipid is naturally very active in bone marrow in accordance with active cell division.

Materials and methods

Male rats of Sprague-Dawley strain weighing 130–150 g were used. Fraction 3 and 4 of the extract from the roots of ginseng were kind gifts from Prof. Oura. Ginsenosides Rb_1, b_2, c, e and g_1 were kind gifts from Prof. Shibata and Prof. Shoji.

In the *in vivo* experiments, fraction 3 was orally administered daily for one to two weeks. In some experiments, fraction 4 was injected intraperitoneally 3 or 6 hours prior to the sacrifice. In the other experiments, fraction 4 was directly added to the incubation medium. Synthesis of DNA, RNA, protein and lipid were determined by the incorporation of thymidine-^{3}H, uridine-^{3}H, l-leucine-^{14}C and acetate-1-^{14}C into DNA, RNA, protein and lipid, respectively *in vitro*. Bone marrow was scraped out from long bones. Bone marrow cells were dispersed using a glass homogenizer, washed several times by medium 199. The cell suspension was placed in tubes containing medium 199, pH 7.4, to which the radioactive precursor was added.

Incubation was carried out for 1 hour in 95% O_2-5% CO_2 at 37°C. DNA and RNA were extracted and purified according to the method of Miura[8] using SDS and phenol. Protein was precipitated by TCA, washed by TCA and acetone. Total lipid was extracted with chloroform-methanol. (2:1), washed with water and subjected to TLC.[9][10] Radioactivity was determined by a liquid scintillation counter. Quenching was corrected.

Ginsenosides were used in the same way. In the *in vivo* experiments, ginsenosides were intraperitoneally injected 4 hours prior to the sacrifice.

Cyclic AMP was determined by the method of Kumon et al.[11]

Results

Oral administration of fraction 3 increased lipid, protein and DNA synthesis. Direct addition of

Effect of oral administration of Panax extract Fraction IV on DNA, protein and lipid synthesis in bone marrow cells

	Total lipids	Cholesterol	Fatty acids	Phospholipids	Protein	DNA
	mμmoles of each precursor incorporated/h/3.0 × 10^7 cells					
Control	2.73 ± 0.29*	0.15 ± 0.02	0.42 ± 0.06	0.86 ± 0.08	2.90 ± 0.16	1.17 × 10^{-1}±0.12
Panax extract Fraction IV 1 mg/100 g b. w./day × 7 days oral	5.52 ± 0.38	0.38 ± 0.05	0.97 ± 0.16	1.53 ± 0.24	4.20 ± 0.44	1.69 ± 0.14
	P < 0.001	P < 0.001	P < 0.01	P < 0.001	P < 0.001	P < 0.02

Bone marrow cells were incubated in 2 ml of medium 199 containing acetate-1-^{14}C, 1-leucine-U-^{14}C or thymidine-methyl-^{3}H for 1 hour at 37°C in 95 % O_2-5% CO_2.
* Mean ± standard error No.: 8

Effect of *in vitro* addition of Panax extract Fraction IV on DNA, protein and lipid synthesis in bone marrow cells

	Total lipids	Cholesterol	Fatty acids	Phospholipids	Protein	DNA
	mμmoles of each precursor incorporated/h/3.5 × 10^7 cells					
Control	2.51 ± 0.13*	0.11 ± 0.01	0.31 ± 0.01	0.63 ± 0.04	3.42 ± 0.41	1.26 × 10^{-1}±0.13
Panax extract Fraction IV						
10 g/ml	2.89** ± 0.17	0.12** ± 0.01	0.35 ± **0.02	0.72** ± 0.06	4.27** ± 0.35	2.34+ ± 0.28
100 g/ml	3.33+ ± 0.15	0.15*** ± 0.01	0.42+ ± 0.02	0.82*** ± 0.05	5.11*** ± 0.48	2.15+ ± 0.20
1000 g/ml	1.97** ± 0.29	0.09** ± 0.01	0.25*** ± 0.02	0.52** ± 0.03	2.60** ± 0.23	0.91** ± 0.10

* Mean ± standard error No.: 6
** Non-significant
*** P < 0.05
\+ P < 0.01
Bone marrow cells were incubated in 2 ml of medium 199 containing acetate-1-14C, 1-leucine-U-14C or thymidine-methyl-3H for 1 hour at 37°C in 95%O_2-5%CO_2.

Effect of *in vitro* addition of Panax extract Fraction IV on DNA synthesis in bone marrow cells.
Thymidine incorporated into DNA

	No. of incubation	$\mu\mu$moles/h/2 $\times$ 10^7 cells	
Control	6	50.3 $\pm$ 6.8*	
Panax extract			
Fraction IV			
1 μg/ml	6	55.2 $\pm$ 6.0	N. S.**
5	6	77.3 $\pm$ 6.7	$P < 0.05$
10	6	91.5 $\pm$ 5.9	$P < 0.01$
25	6	105.5 $\pm$ 8.0	$P < 0.001$
50	6	98.0 $\pm$ 8.4	$P < 0.01$
100	6	86.9 $\pm$ 5.1	$P < 0.01$
250	6	74.0 $\pm$ 9.0	N. S.
500	6	57.0 $\pm$ 7.4	N. S.
1000	6	39.0 $\pm$ 6.0	N. S.

* Mean $\pm$ standard error
**Non-significant
Bone marrow cells were incubated in 2 ml of medium 199 containing thymidine-methyl-3H (2.5 μCi, 20 mμmoles) for 1 hour at 37°C in 95% O_2-5% CO_2.

Effect of cycloheximide pretreatment on stimulatory effect of ginseng extract fraction 4 *in vitro* on DNA and protein synthesis in rat bone marrow.

	DNA	P&OCEIN
	mμmoles of each precursor incorporated/ h/2.8 $\times$ 10^7 cells	
Non-treated rats		$\times 10^{-1}$
Control	1.01 $\pm$ 0.11*	2.56 $\pm$ 0.23
Fraction 4	1.64 $\pm$ 0.19***	3.85 $\pm$ 0.33***
Cycloheximide-treated		
Control	0.26 $\pm$ 0.05	1.18 $\pm$ 0.15
Fraction 4	0.30 $\pm$ 0.04**	1.57 $\pm$ 0.14**

*Mean $\pm$ standard error, **non-significant, ***$p < 0.05$
No.: 6
Fraction 4 was added to the incubation medium to the concentration of 50 μg/ml. Cycloheximide was injected intraperitoneally 4 hours prior to the sacrifice (0.1 mg/100 g B. W.).

fraction 4 *in vitro* stimulated DNA synthesis. Protein and lipid synthesis was also slightly increased.

Pretreatment of cycloheximide 4 hours before sacrifice much reduced the stimulatory effect of fraction 4 which was added to the incubation medium on DNA and protein synthesis. Ginseng principles might have the action of enzyme induction which was involved in DNA, portin and lipid synthesis.

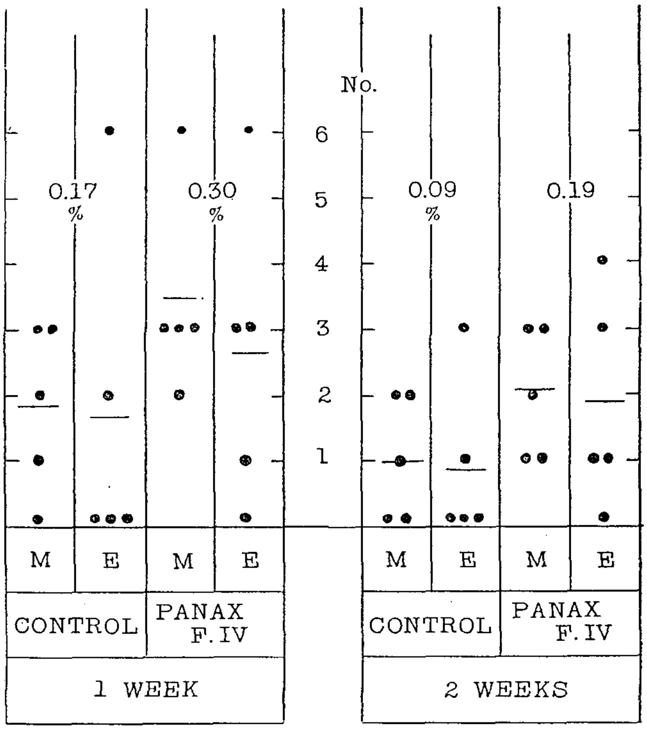
The stimulatory action on DNA synthesis *in vitro* was also observed using human bone marrow.

Effect of panax ginseng extract on DNA & RNA synthesis *in vitro* by rat bone marrow

Time after panax inject. hr(s) (No.)	DNA synthesis from thymidine	RNA synthesis from uridine
	mμ moles/hr	
0	(5) 0.024 $\pm$ 0.003	0.106 $\pm$ 0.012
3	(5) 0.046 $\pm$ 0.005 $p < 0.001$	0.276 $\pm$ 0.032 $p < 0.01$
6	(5) 0.045 $\pm$ 0.005 $p < 0.001$	0.225 $\pm$ 0.025 $p < 0.01$
		mean $\pm$ S.E.

Thymidine-methyl-H^3 2.5μC 20mμmoles
Uridine-U-H^3 5 μC 20 μmoles
1.8 $\times$ 10^7 cells

Effect of oral administration of Panax Fraction IV (1 mg/100 g b. w./day) on numbers of mitosis in bone marrow



Numbers of mitosis among 2,000 nucleated cells 5 hours after colchicin injection (0.1 mg/100 g i. p.)

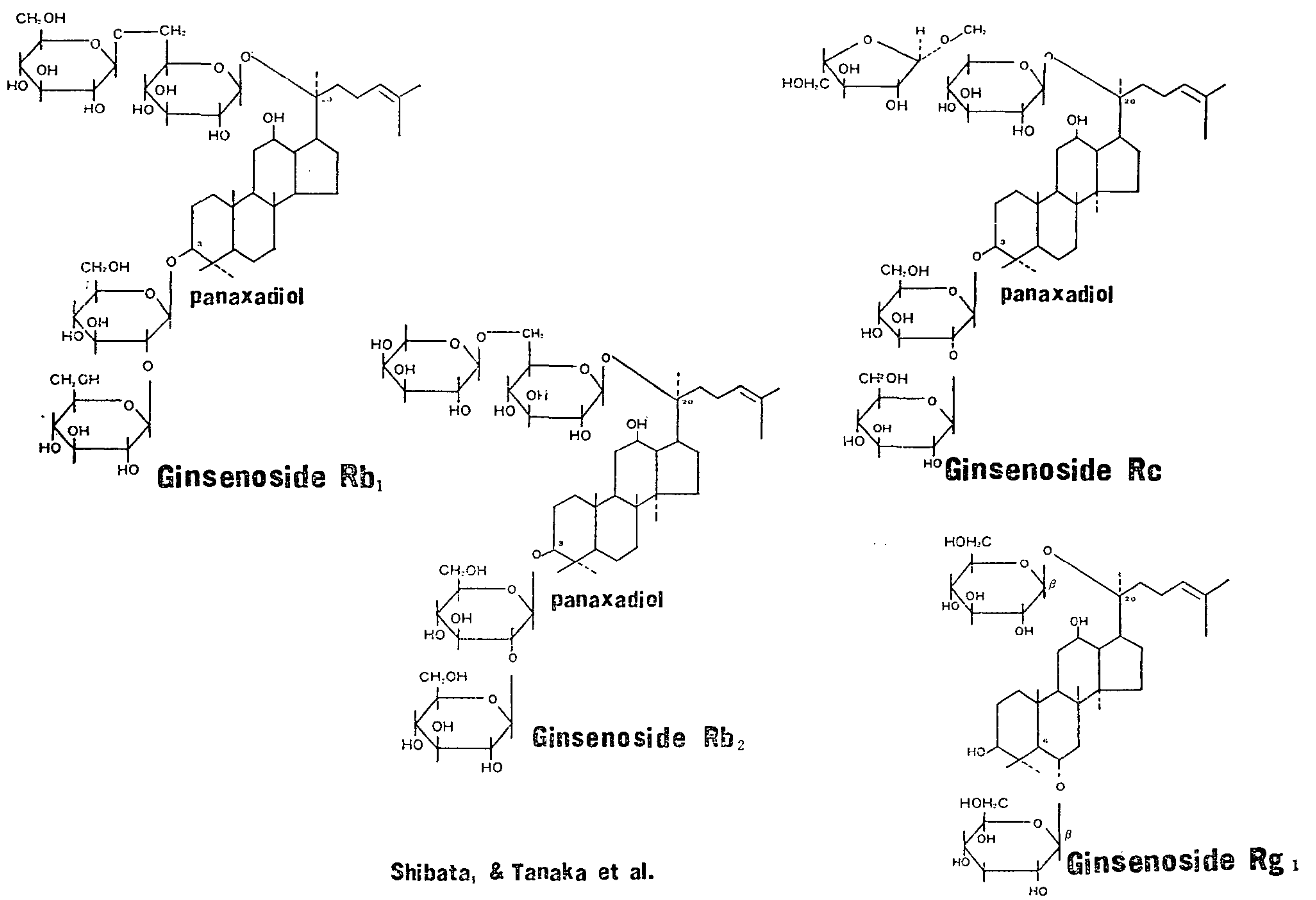
Single intraperitoneal injection of fraction 4 enhanced DNA and RNA synthesis 3 and 6 hours later.

As to morphological examinations, oral administration of fraction 3 for one and two weeks increased numbers of mitosis in bone marrow. The rate of increase by ginseng in numbers of mitosis was almost equal in both myeloid and erythroid. From this fact and the result from the *in vitro* experiments, ginseng action might not be associated with erythro-

Effect of oral administration of ginseng extract fraction 3 on hematological findings.
Fraction 3: 1 mg/100 g body weight/day for 7 days

PERIFERAL BLOOD	Change of B. W. (g)	W. B. C. (/cmm)	R. B. C. ($\times 10^4$/cmm)	Hb (g/dl)	Ht (%)	Reticulo. (%)	
Non-treated*	+ 48(188 → 236)	6,900	591	12.1	45.4	14 ± 1.6**	
Fraction 3*	+ 58(192 → 250)	7,640	606	12.3	46.8	23 ± 2.5** ($p < 0.05$)	
BONE MARROW	**Total nucleated cells (/cmm)**	**Mbl Mpro**	**M Mmeta**	**St**	**Seg**	**Ly**	**Ebl**
Non-treated*	1.20 ± 0.17**	5	31	17	12	9	26
Fraction 3*	1.86 ± 0.11** ($p < 0.05$)	7	32	16	10	7	28

* No. of animals: 10, ** Mean ± standard error, $p < 0.05$



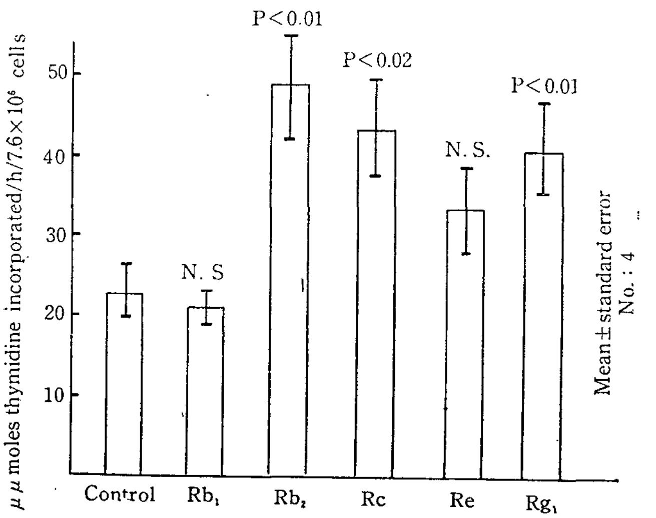
poietin. Also cell analysis revealed no change in the ratio of bone marrow cells, while an increase was seen in blood reticulocytes and total nucleated cells in bone marrow.

These results mentioned above might give some biochemical basis to the hematopoietic action of ginseng and make way to the clinical application of ginseng to anemias and leucopenia.

Chemical structure and actions of ginseng principles

Several kinds of saponins, named ginsenosides, had been purified and structurally determined by Prof. Shibata and his colleagues, as mentioned before. As we assumed that some of these ginsenosides should be the major active principles in roots of ginseng, we studied the effects of ginsenosides on DNA, protein and lipid synthesis in bone marrow.

Effect of intraperitoneal injection of ginsenoside Rb_1, b_2, c, e & g_1 on DNA synthesis in bone marrow cells



One mg per 100 g b. w. of each was injected intraperitoneally 4 hours before sacrifice.

Bone marrow cells were incubated in 2 ml of medium 199 containing thymidine-methyl-^3H (5μCi, 40 mμmoles).

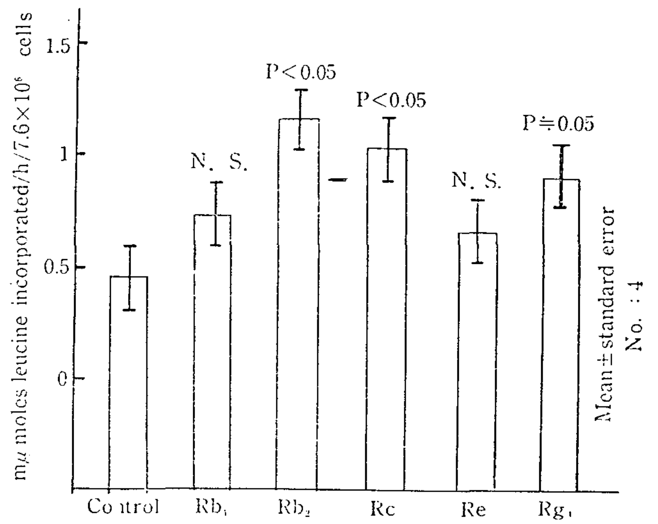
The intraperitoneal injection of ginsenosides Rb_1, b_2, c, e, and g_1 was tried. DNA synthesis was significantly increased by the administration of ginsenosides Rb_2, c and g_1, while no significant increase was observed by Rb_1, and e treatment. Almost the same tendency was obtained in protein and lipid synthesis.

Mixture of Rb_1, b_2 and c (GNS) was' added to the incubation mixture. Significant increase in the DNA synthesis was seen at the concentration of 0.1–1 μg/ml.

It is conceivable that the stimulatory action of ginseng *in vivo* and *in vitro* was derived from ginsenosides at least in part.

Here, I would like to talk about chemical structure and action of these ginsenosides. Either of Rb_1,

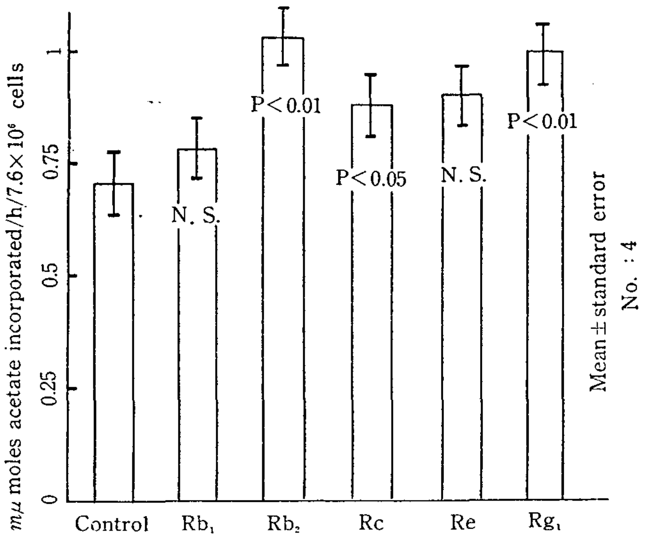
Effect of intraperitoneal injection of ginsenoside Rb_1, b_2, c, e & g_1 on protein synthesis in bone marrow cells



One mg per 180 g b. w. of each was injected intraperitoneally 4 hours before sacrifice.

Bone marrow cells were incubated in 2 ml of medium 199 containing 1-leucine-U-14C (1 μCi, 2.6 μmoles).

Effect of intraperitoneal injection of ginsenoside Rb_1, b_2, c, e & g_1 on total lipid synthesis in bone marrow cells

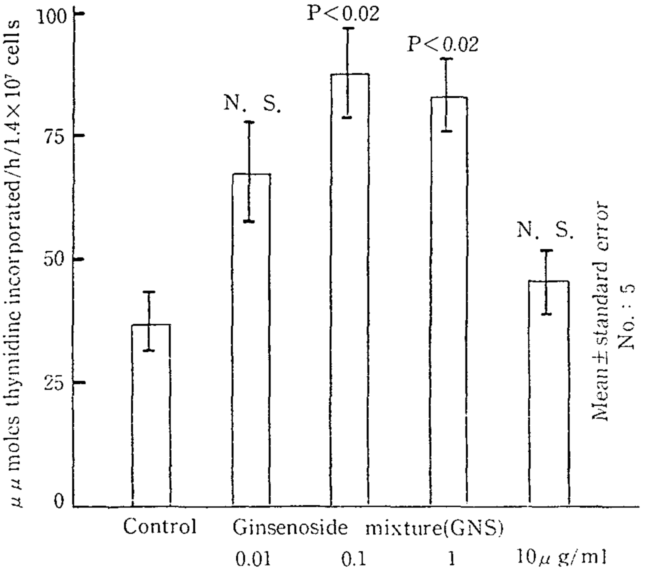


One mg per 100 g b. w. of each was injected intraperitoneally 4 hours before sacrifice.

Bone marrow cells were incubated in 2 ml of medium 199 containing sodium acetate-1-14C (5μ Ci, 3.2 μmoles).

b_2 or c has panaxadiol as their common sapogenin. If b_2 and c were active and b_1 was relatively inactive, arabinose-glucose at 20 position should be important in the biological action of ginsenosides of panaxadiol series. The common sapogenin of ginsenosides Re

Effect of *in vitro* addition of ginsenoside mixture on DNA synthesis in bone marrow cells



Bone marrow cells were incubated in 2 ml of medium 199 containing thymidine-methyl-^{3}H (5 μCi, 40 mμmoles).

and g_1 is panaxatriol. Re has rhamnose-glucose at 6 position, and glucose at 20 position, while Rg_1 has only glucose at 6 position. Of course, panaxadiol and panaxatriol are essential for the biological action of ginsenosides. It is possible that sugar portion of ginsenoside molecule may modify its action. As we can change the type and strength of biological actions of steroids and bile acids, we might get new types of saponins by modifying chemical structure.

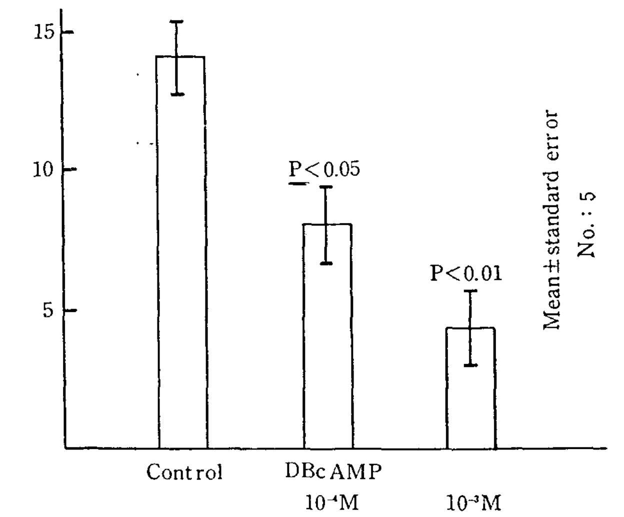
Participation of cyclic AMP in ginsenoside action

Many evidences were reported which show that cyclic AMP was the 2nd messenger of hormonal actions, especially of peptide hormones and catecholamines. It is interesting to know the relationship between cyclic AMP and bone marrow function and to clarify the possible participation of cyclic AMP in the stimulatory action of ginsenosides on bone marrow.

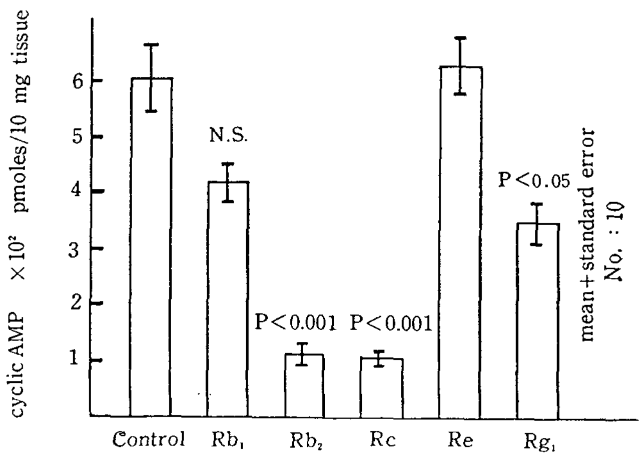
At first, effect of a direct addition of dibutylyl cyclic AMP *in vitro* on DNA synthesis in bone marrow was investigated. DNA synthesis in bone marrow was significantly decreased by an addition of dibutylyl cyclic AMP to the incubation medium at the concentration of 10^{-4} to 10^{-3} M.

Cyclic AMP levels in bone marrow cells were

Effect of Direct Addition of Dibutylyl Cyclic AMP on DNA Synthesis in Bone Marrow Cells



Effect of Intraperitoneal Injection of Ginsenosides Rb_1, Rb_2, Rc, Re, & Rg_1 on Cyclic AMP Levels in Rat Bone Marrow cells



0.5 mg/100 g body weight 20 min Prior to Sacrifice

significantly dcreased by a single intraperitoneal injection of ginsenosides Rb_2, c or g_1 20 minutes prior to the sacrifice, but no significant decrease was observed by ginsenosides Rb_1 and e.

These data may show the participation of cyclic AMP in the stimulatory action of DNA synthesis in bone marrow by ginsenosides. But, further investigation should be necessary.

Biochemical actions on testes

As mentioned before, DNA, protein and lipid synthesis in bone marrow was stimulated by ginseng principles. In the liver, where DNA synthesis is not active, DNA was not increased by ginseng pinciples, as Dr. Hiai reported. We started the following experiments, assuming that DNA synthesis may be stimulated by ginseng principles in the testes where DNA synthesis is active.

We studied the change in DNA and protein synthesis by the *in vitro* addition of fraction 4 (Prof. Oura) to the incubation medium in which minced testis was placed. DNA synthesis as well as protein synthesis was increased by addition *in vitro* of fraction 4. Cycloheximide pretreatment reduced the stimulatory action of fraction 4 on DNA and protein synthesis. It is conceivable, therefore, that ginseng principles may induce the enzymes involved in DNA and protein synthesis.

Effect of addition of ginseng extract fraction 4 *in vitro* on DNA and protein synthesis in minced testes of rats.

	DNA	PROTEIN
	mμmoles of each precursor incorporated/h/100 mg tissue	
		$\times 10^{-1}$
CONTROL	0.64 ± 0.05**	2.33 ± 0.17*
FRACTION 4		
10 μg/ml	0.97 ± 0.09***	2.81 ± 0.35**
50 μg/ml	1.13 ± 0.16***	3.16 ± 0.26***

* mean ± standarderror, ** non-significant,
*** $p < 0.05$ No.:8

Effect of cycloheximide pretreatment on stimulatory action of ginseng extract fraction 4 *in vitro* on DNA and protein synthesis in rat testes.

	DNA	PROTEIN
	mumoles of each precursor incorporated/h/100 mg tissue	
Non-treated rats		$\times 10^{-1}$
Control	0.47 ± 0.06*	2.15 ± 0.18*
Fraction 4	0.98 ± 0.15***	2.99 ± 0.26***
Cycloheximide-treated		
Control	0.13 ± 0.01	1.09 ± 0.17
Fraction 4	0.16 ± 0.04**	1.12 ± 0.09**

*Mean ± standard error, **non-significant, ***$p < 0.05$
No.:6

Fraction 4 was added to the incubation medium up to the concentration of 50 μg/ml. Cycloheximide was injected intraperitoneally 3 hours prior to the sacrifice (0.2 mg/100 g body weight).

These results might offer the evidence about spermatogenesispromoting action of ginseng which was observed by Prof. Shida and Prof. Ishigami.

Conclusion

1) Effects of fractions 3 and 4 from ginseng and ginsenosides on bone marrow and testes were investigated.

2) Fraction 3 and fraction 4, *in vivo* and *in vitro* stimulated DNA, RNA, protein and lipid synthesis in bone marrow, as well as testes.

3) Cycloheximide pretreatment reduced stimu-, latory effect of fraction 4 on DNA, protein and lipid synthesis in bone marrow and testes.

4) Mitotic indices were increased in myeloid and erythroid by administration of fraction 3, as well as reticulocytes and total nucleated cells.

5) Ginsenosides Rb_2, c and g *in vivo* and GNS *in vitro* increased DNA, protein and lipid synthesis in bone marrow but Rb_1 and e did not.

6) Ginsenosides Rb_2, c and g_1 *in vivo* decreased cyclic AMP levels and dibutylyl cyclic AMP *in vitro* inhibited DNA synthesis in bone marrow cells.

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