EFFECT OF RED GINSENG POWDER ON LIPOLYTIC AND ANOREXIGENIC FACTOR (TOXOHORMONE-L) FROM CANCEROUS ASCITES FLUID

Hiromichi Okuda, Hiroshi Masuno, and Song Jae Lee
2nd Department of Medical Biochemistry, School of Medicine, Ehime University, Japan
** Korea Ginseng & Tobacco Research Institute, KOREA*

ABSTRACT

Toxohormone-L (THL) elicited fatty acid release from rat epididymal adipose tissue, which is present in cancerous ascites fluids. In this study, the effect of ginseng powder on lipolysis induced by Toxohormane-L, and ACTH was studied. Korean ginseng selectively inhibited Toxohormone-L induced lipolysis, but did not inhibit ACTH-induced lipolysis.

INTRODUCTION

During progressive weight loss in patients with various neoplastic disease, depletion of fat stores have been observed. The depletion of body fat during growth of neoplasms is associated with increase in plasma free fatty acids.

Recently, we found that the ascites fluid from patients with hepatoma or ovarian tumor and the pleural fluid from patients with malignant lymphoma elicited fatty acid release in slices of rat adipose tissue in vitro, The lipolytic factor, named "toxohomone-L", was purifed from the ascites fluid of patients with hepatoma. The isolated preparation gave a single band on both disc gel electrophoresis and sodium dodecyl sulfate(SDS)-acrylamide gel electrophoresis in the presence of β-mercaptoethanl. Its molecular weight was determined to be 70,000-75,000 and 65,200 by SDS-acrylamide gel electrophoresis and analytical ultracentrifugation, respectively.

Injection of toxohormone-L into the lateral ventricle of rats significantly suppressed food and water intakes. There was at least 5 hrs delay between its injection and appearance of its suppressive effect.

In this study, we tried to find a inhibitory substance toward toxohormone-L from root powder of red ginseng. An inhibitory substance toward lipolytic action of the toxohormone-L was demonstrated in water extract of red ginseng. The inhibitory substance was contained in saponin free fraction of the red ginseng extract.

MATERIALS AND METHODS

1. Materials

Red ginseng powder (6 years old) was extracted by 5 volumes of distilled water and the extract was subjected to dialysis against water. The inner dialysate was treated with petroleum, chloroform and butanol (saturated with water), successively, to remove lipids and saponins. The resultant non-saponin fraction was treated with active carbon and then applied to DEAE-cellulose column. By these procedures, an active fraction (red ginseng fraction 3) was prepared.

Animals.

Male wistar strain rats, weighing 150-180g, were used. They were given standard laboratory diet and water ad libitum.

Preparation of Ascites fluid.

The mice were inoculated i.p with 0.5ml of sarcoma 180 suspension (4 to 5 x 10^9 cells/mouse), and 10 to 14 days later, the ascites fluid was harvested. And another ascites fluids were obtained from several hepatoma patients. The ascites fluid was centrifuged at 1000 x g for 10min at 4°C to remove cell debris.

Measurement of Body Triglyceride.

Whole mice were minced in a mixer at 0°C in 10 volumes of extraction mixture (n-hexane: ether: ethanol (5:5:2, v/v). The homogenate was centrifuged at 1000 x g for 10 min at 0°C. The supernatant was collected, concentrated in vaccum, and dried over $CaCl_2$ for 12hr. The triglyceride content was measured with wako triglyceride B-test.

Estimation of Lipolytic Activity of Toxohormone-L.

Male rats were sacrificed by a blow on the head, and their epididymal adipose tissue was removed quickly. Then, 100mg of minced adipose tissue were incubated in a glass-stoppered test tube for 2 hr at 37°C with 1ml of Krebs-Ringer bicarbonate buffer (pH 7.4) containing 2.5% bovine albumin and 2.5 x 10^{-3}M calcium chloride in the presence of toxohormone-L. After incubation, FFA was extracted and titrated with NaOH as described by Dole.

RESULTS AND DISCUSSION

Body Triglyceride Contents of Control and Tumor-bearing mice.

Change of triglyceride content was compared between control and Sarcoma 18-bearing mice. As shown in Fig. 1. Triglyceride content of the tumor-bearing mice was considerably decreased as compare to control mice.

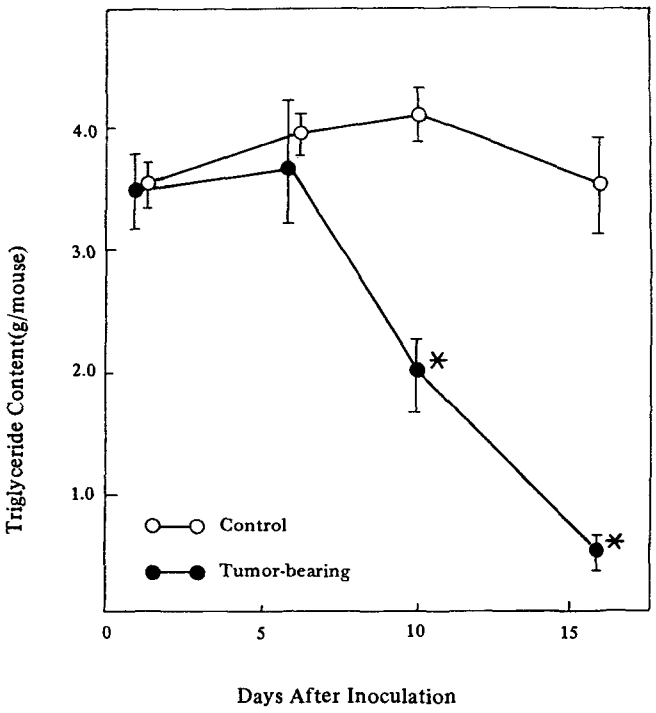

Fig. 1. Change of triglyceride content in control and tumor bearing mice.

Food Intake of Control and Tumor-Bearing Mice.

After inoculation of Sarcoma 180, food intake was remarkably reduced in tumor-bearing mice as compare to that of control mice.

Therefore, it seems likely that reduction of triglyceride content in tumor-bearing mice might be partly due to decrease of food intake in these animals (Fig. 2). However, we found that another mechanism exists for the reduction of triglyceride content in tumor-bearing animals.

Lipolytic Factor from Cancerous Ascites Fluid.

We found a lipolytic factor in ascites fluid. We found a lipolytic factor in ascites fluid from patients with hepatoma. When the epididymal adipose tissue slices were incubated with the cancerous ascites fluid, remarkcable release of free fatty acid was found (Fig. 3). On the other hand, ascites fluid from patients with liver cirrhosis did not show such a lipolytic activity. The lipolytic factor was found in cancerous ascites fluid from mouse Sarcoma 180, human ovarian tumor and human Grawitz's tumor in addition to human hepatoma. However, there was no lipo-

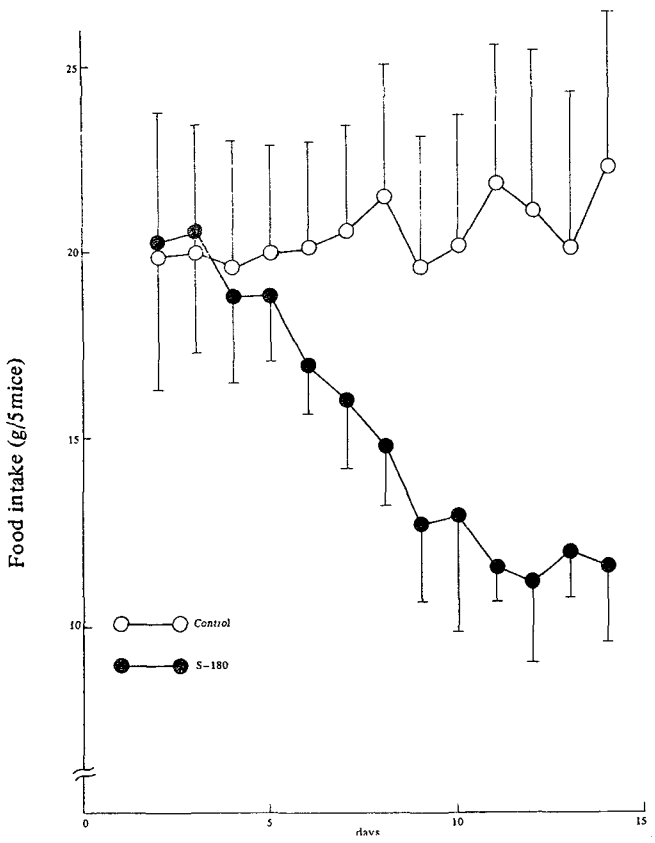

Fig. 2. Food intake of tumor-bearing and control mice.

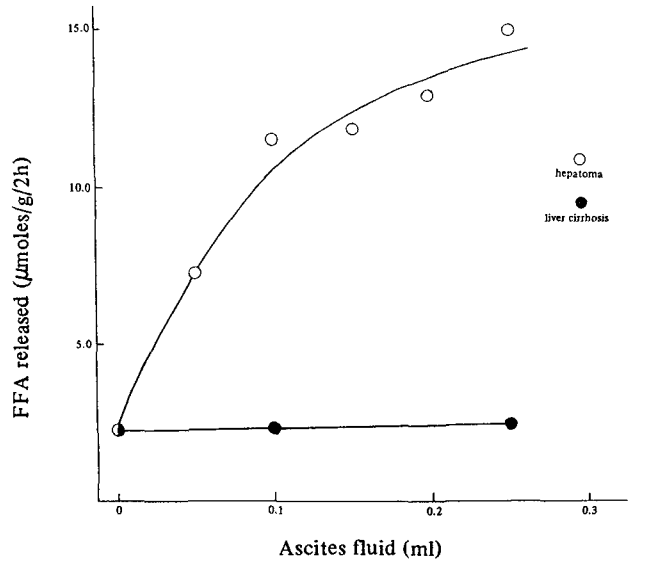

Fig. 3. Lipolytic activity of ascites fluid in adipose tissue slices.

lytic factor in non cancerous ascites fluid from mouse peritenitis and human liver cirrhosis (Table 1.). We termed the lipolytic factor "toxo hormone'L" and tried to isolate and characterite it from the ascites fluid of patients with hepatoma.

Isolation of Toxohormone-L.

The ascites fluid was collected from patients with hepatoma and subjected to ammonium sulfate fractionation, DEAE-cellulose column chromatography, blue sepharose column chromatography and chromatofocusing column chromatography (Table 2.). In this way, 5.6% of the activity of toxohormone-L was recovered from the ascites fluid. As shown in Fig. 4. Four main peaks were eluted and toxohormone-L activity showed an isoelectric point of 4.7 to 4.8. The purified toxohormone-L gave a single band on disc gel electrophoresis and SDS-acrylamide gel electrophoresis in the presence of β-mercaptoethanol(Fig. 5). The molecular weight of toxohormone-L was estimated at 70,000 - 75,000 on SDS-acrylamide gel electrophoresis. On sedimentation analysis, toxohormone-L sedimented as a single peak with a sedimentation coefficient of 4.81S. The weight-average molecular weight calculated from the sedimentation equilibrium data was 65,200. The N-terminal amino acid was found to be aspartic acid(Table 3.).

Table 1. Existance of toxohormone-L in the Ascites Fluids obtained from Patients and Mice with Various Diseases

	Source of ascites fluid	Lipolytic activity (units/mg protein)	
I.	cancerous ascites fluid		
	mouse sarcoma 180	2.7 ± 0.3	(12)
	human hepatoma	7.2 ± 1.7	(4)
	human malignant lymphoma*	7.7 ± 1.0	(4)
	human ovarian tumor	7.3 ± 0.3	(3)
	human Grawitz's tumor	3.3	(2)
II.	noncancerous ascites fluid		
	mouse peritonitis	0.0	(2)
	human liver cirrhosis	0.0	(2)
	human alcoholic liver cirrhosis	0.0	(2)

The Effect of Toxohormone-L on Food intake and Water intake.

The anorexigenic effect of toxohormone-L

Table 2. Isolation of Toxohormone-L from the Ascites Fluid of Patients with Hepatoma

Isolation step	Total protein (mg)	Total activity (units)	Yield (%)	Specific activity (units/mg)	Purification (fold)
Ascites fluid	1125.0	10800	100.0	9.6	1.0
40-70% $(NH_4)_2$ SO_4, ppt	138.7	8800	81.5	63.4	6.6
DEAE-cellulose column	12.0	3325	30.8	277.1	28.9
Blue sepharose column	8.2	2531	23.4	308.4	32.2
1st chromatofocusing column	2.6	1144	10.6	440.0	45.8
2nd chromatofocusing column	0.8	604	5.6	755.0	78.6

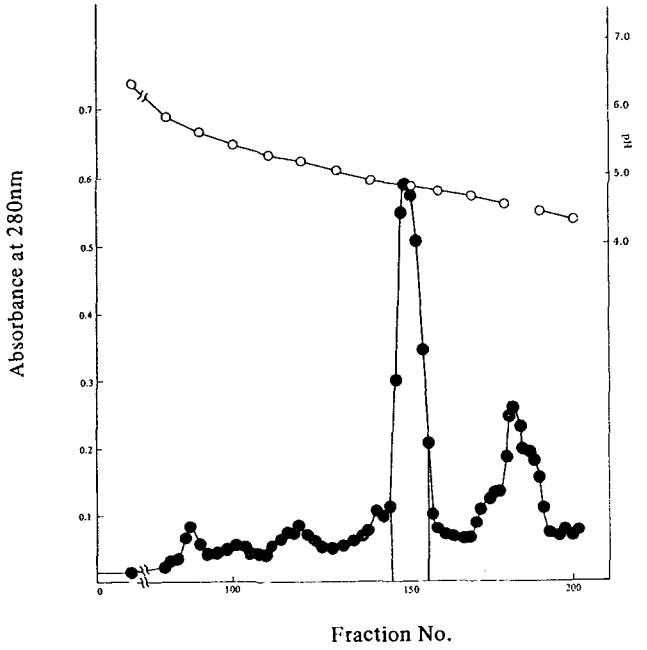

Fig. 4. Chromatography of toxohormone-L in the ascites fluid of patients with hepatoma on a chromatofocusing column.

Table 3. Molecular Weight of Toxohormone-L in the Ascites Fluid of Patients with Hepatoma

	Molecular Weight
SDS-polyacrylamide gel electrophoresis	70,000 – 75,000
Sedimentation equilibrium	65,200 (S=4.81s)

was studied in a group of 7 rats that had been trained to ingest all their food in a 2hr period each day. As shown in Fig. 6-A, B, injection of toxohormone-L into the lateral ventricle resulted in significant suppression of food intake in a dose dependent manner. For determination of the specificity of the effect of toxohormone-L, the effect of bovine albumin was examined. Rats injected with 14.4ug of bovine albumin exhibited no significant alternation in food or water intake.

From above results, toxohormone-L secreted from tumor tissue exhibited dual actions on the host. One is increase of lipolysis in the adipose tissue and the other is induction of anorexia. These actions of toxohormone-L results in loss of body fat in cancer patients (Fig. 7.).

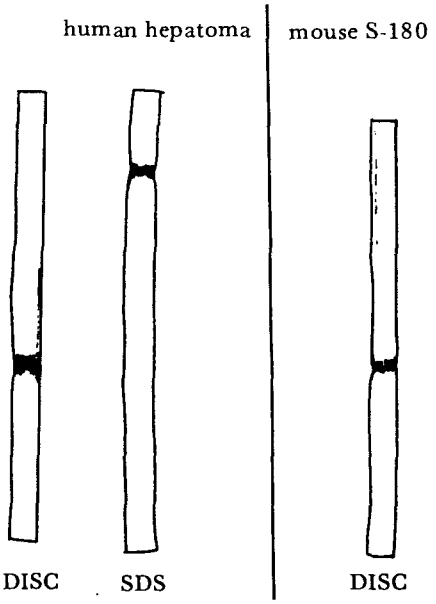

Fig. 5. DISC gel electrophoresis and SDS-acrylamide gel electrophoresis of toxohormone-L.

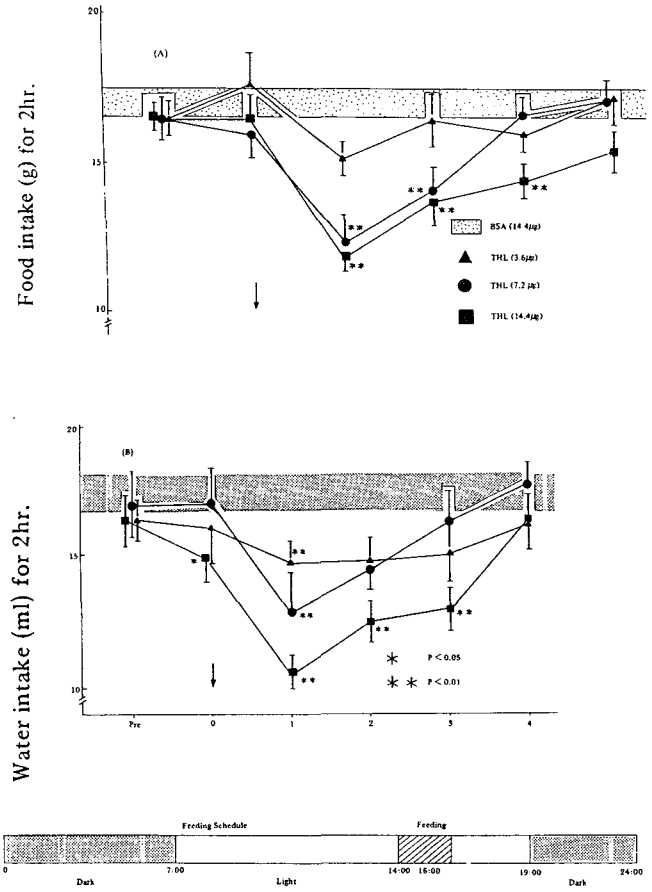

Fig. 6-A, B. Effect of intraventricular injection of toxohormone-L on food intake(A) and water intake (B).

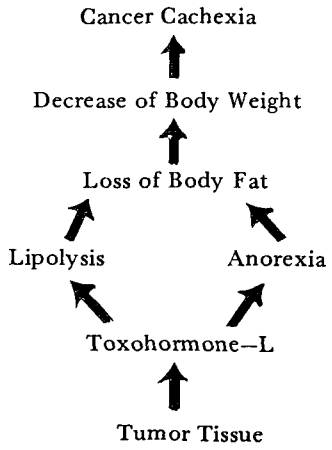

Fig. 7. Toxohormone-L actions on the host.

The Effect of Red Ginseng Powder on Toxohromone-L.

It is well known that administration of red ginseng to cancer patients improves anorexia and protects decrease of the body weight. Therefore, we designed the experiments to clarify whether or not red ginseng contained some inhibitory substances toward toxohormone-L.

Isolation of Inhibitory Substance from Red Ginseng.

Red ginseng powder was extracted with water and subjected to dialysis against water. The inhibitory substance toward toxohormone-L was found in the inner dialysate. The inner dialysate was then treated with butanol saturated with water. The resultant non-saponin fraction was found to inhibit a lipolytic activity of toxohormon-L. Then, the non-saponin fraction was applied to active carbon column and eluted with 10%, 20% and 50% ethanol, successively. The inhibitory substance toward toxohormone-L was found in 50% ethanol eluate. The 50% ethanol eluate was then applied to DEAE-cellulose column. The inhibitory substance was absorbed on this cellulose and found in Fraction III (Fig. 8.).

Toxohormone-L-inhibiting Activity of Inner Dialysate.

As shown in Fig. 9, toxohormone-L-inhibit-

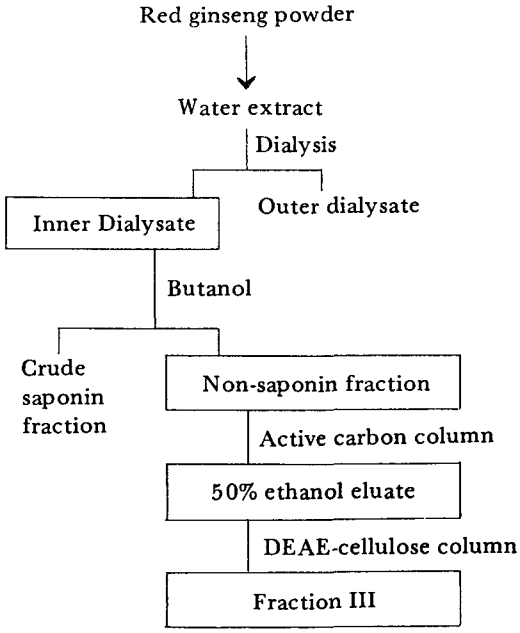

Fig. 8. Isolation of inhibitory substance toward toxohormone-L from Red ginseng powder.

ing activity was found in non-saponin fraction in the inner dialysate. However, crude saponin fraction has no effect on toxohormone-L-inhibiting activity.

Column Chromatography of Inner Dialysate.

Fig. 10 is the DEAE-cellulose column chromatography of 50% ethanol eluate from active carbon column. The absorbed material on this column was eluted with NaCl. Solid line indicates the pattern of carbohydrate estimated with phenol sulfuric acid. The dotted line shows the pattern of protein measured by the absorption at 280nm. Toxohormone-L-inhibiting activity was found in Fraction III, suggesting that the inhibitory substance might be a polysaccharide.

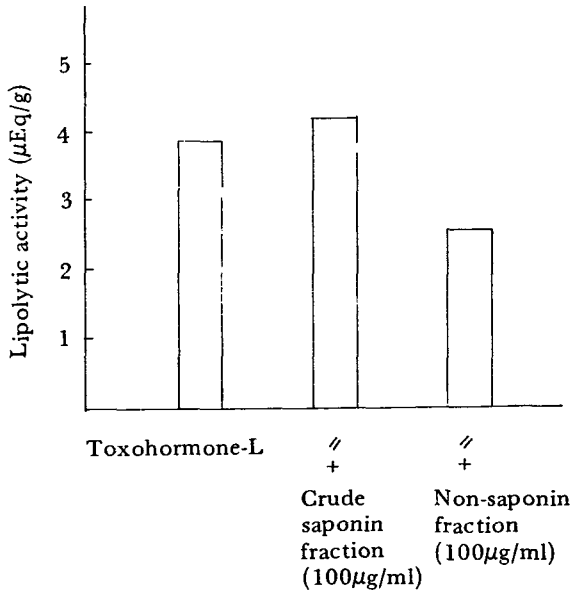

Fig. 9. Toxohormone-L-inhibiting activity in inner dialystate.

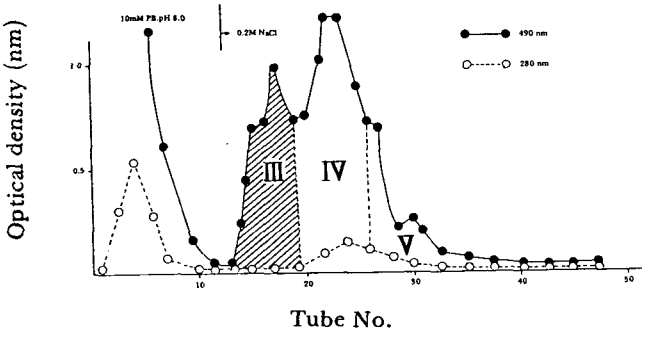

Fig. 10. DEAE-cellulose column chromatography of 50% ethanol eluate from active carbon column.

Effect of Fraction III on Toxohormone-L and ACTH-induced Lipolysis.

Fraction III, as shown in Fig. 11, did not inhibit ACTH-induced lipolysis, but selectively reduced toxohormone-L-induced lipolysis. Therefore, it follows that the inhibitory substance from red ginseng did not exert any action on physiological hormone, but inhibit selectively toxohormone-L activity.

From these results, as summerized in Fig. 12 loss of body fat in cancer patients might be induced by dual actions of toxohormone-L, stimulation of lipolysis in adipose tissue and induction of anorexia and red ginseng might protect loss of body fat by inhibiting these actions of toxohormone-L.

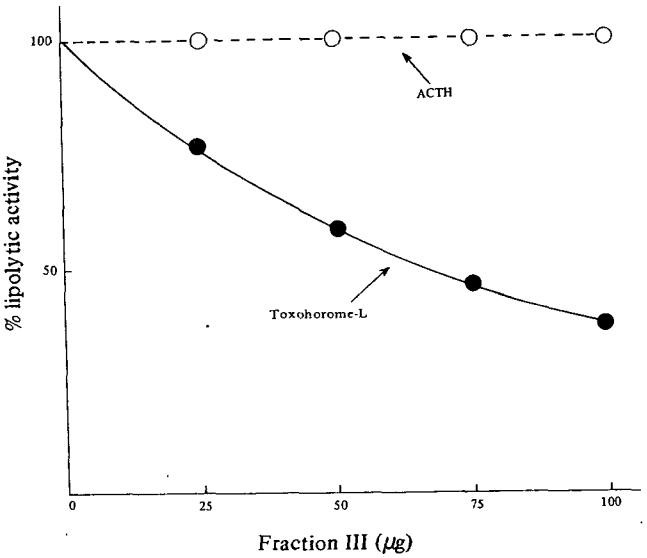

Fig. 11. Effect of fraction III on toxohormone-L and ACTH-induced lipolysis in adipose tissue slices.

Biological Active Substnaces in Red Ginseng.

Our experiments on Red ginseng summaried in Fig. 13. We found several biological active substances in red ginseng. The inner dialysate of the water extract from red ginseng powder contains toxohormone-L-inhibiting substance. In the previous ginseng symposium, I reported that the outer dialysate of the water extract possessed some selective modulators such as adenosine, Mn-

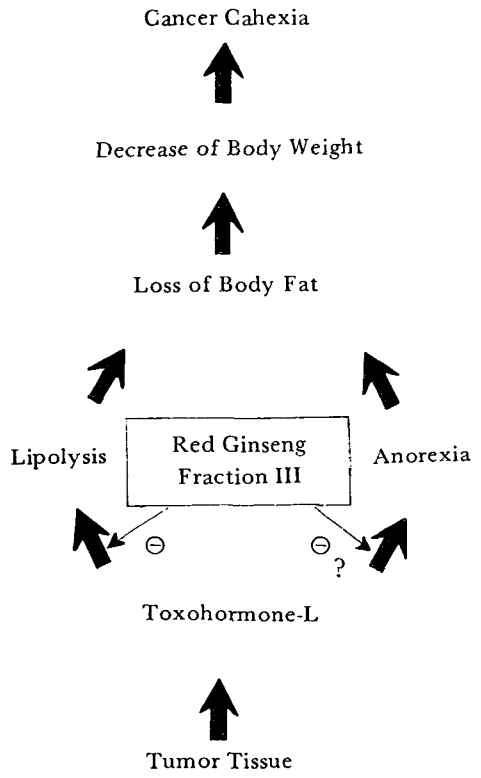

Fig. 12. Inhibitory action of Red ginseng fraction III on toxohormone-L.

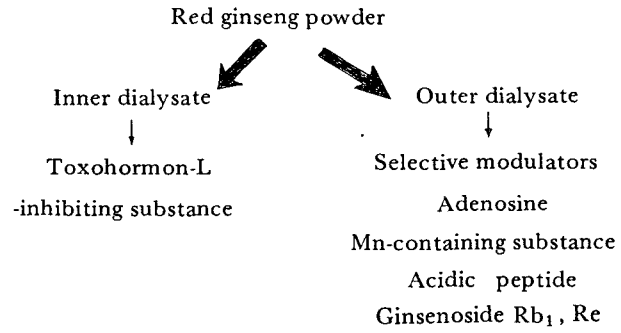

Fig. 13. Summary of Our experiments on red ginseng

contaning substance, an acidic peptide, ginsenoside Rb_1 and Re.

Selective Modulators of Red Ginseng on Insulin Action.

Lipolysis in fat cells is stimulated by insulin antagonists such as adrenaline, noradrenaline, ACTH and GH. On the other hand, as shown in Fig. 14 insulin stimulates the lipogenesis and inhibits the lipolysis. Selective modulators such as adenosine, Mn-containing substance, an acidic peptide, ginsenoside Rb_1 and Re selectively inhibit the actions of insulin antagonists and do not affect but rather stimulate the action of insulin antagonists and do not affect but rather stimulate the action of insulin. In contrast to these selective modulator in red ginseng, a β-blocker, propranolol, inhibited not only the actions of insulin antagonists such as catecholamines, but also the action of insulin. Therefore, it is expected that such selective modulators in red ginseng are more effective than β-blockers as to improving the disturbances of homeostasis.

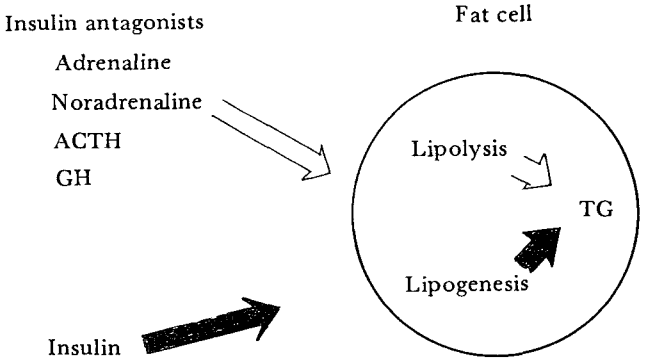

Fig. 14. Selective modulators of Red ginseng on insulin action.

Shibata: The active principle which you isolated is a very high molecular weight compound. Is it possible in oral administration that such a high molecular weight compound can be absorbed and give any effect?

Okuda: We also carried out *in-vivo* experiment with rats. Morris hepatoma was transplanted to the rats and we examined the effect of Red Ginseng Powder on the food intake. At the early stage of the inoculation, oral administration of Red Ginseng Powder actually improved appetite. Professor Nabea, who is a surgeon in Tokyo University, also carried out the clinical experiment using the Red Ginseng Powder and he administered orally to the cancer patients. Other times, anorexia is also improved statistically significantly. So I suppose that active component might have a small molecular weight. I assumed that the active substance might be a polysaccharide. Even if the polysaccharide fraction were degradated

by amylase or something like that, small molecules derived from the polysaccharide fraction might be biologically active.

Chong: How many patietns did you look at? And what happened to your patients?

Okuda: About 70 or so patietns were examined.

암의 복수액에 존재하는 지방분해 및 식욕감퇴 인자에 미치는 고려인삼의 영향

Hiromichi Okuda, Hiroshi Masuno, and Song Jae Lee*
일본 에히메대학 의대 생화학교실
*한국인삼연초 연구소

여러 종류의 종양 환자에서 점차적인 체중 감소가 일어나는 과정에서, 체내에 저장된 지방이 고갈됨이 관찰되었다.

종양이 커가는 동안의 체내 지방의 고갈은 혈장의 유리지방산의 증가와 연관이 있다. 최근에, 우리는 간암, 또는 난소암 환자의 복수액과 악성 임파종 환자의 늑막액은(in vitro에서) 흰쥐의 지방조직에 유리지방산의 방출을 촉진시킴을 발견하였다. Toxohormone-L(독성 호르몬-L)이라고 명명한 이 지방분해 인자를 간암 환자의 복수액에서 분리, 정제하였다.

이 분리된 물질은 disc gel electrophoresis와 β-mercaptoethanol 존재하의 sodium dodecyl sulfate(SDS)-acrylamide gel electrophoresis상에서 단일띠를 나타내었다.

이것의 분자량은 각각 SDS-acrylamide gel electrophoresis와 analytical 초원심분리에 의하여 70,000~75,000과 65,200의 분량을 가짐이 확인되었다.

흰쥐에 Toxohormone-L의 주사는 사료와 물의 섭취를 현저히 억제하였으며, 이 Toxohormone-L의 주사 5시간후에 이러한 억제효과가 나타났다.

Toxohormone-L의 지방분해의 작용을 억제하는 억제물질이 고려인삼 뿌리의 물 추출물에 존재함이 입증되었다.

즉, 인삼 분말을 5배량의 증류수에 현탁시켜 추출하고 감압 농축한 다음 증류수에 투석시켰다. 투석막 안의 지방 성분을 석유 에테르로 제거하고, 사포닌은 수포화된 부탄올로 제거하였다.

이렇게 제거하고 남은 여액을 활성탄으로 처리하고 DEAE-cellulose column에 통과시켰다.

이러한 과정으로 유효한 분획(인삼분획 3)을 얻었으며, 보다 더 순수한 정제가 지금 진행중에 있다.

이러한 실험 결과를 바탕으로 볼 때, 암환자에 고려인삼 분말의 투여는 암조직에서 분비되는 Toxohormone-L(독성 호르몬-L)의 작용을 억제함으로서 암환자의 체중 감소를 방지하고, 식욕감퇴를 개선할 것으로 생각된다.