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STUDIES OF INHIBITORY SUBSTANCE ON LIPOLYTIC FACTOR FROM ASCITES FLUID OF PATIENTS WITH HEPATOMA

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= Abstract =

A lipolytic factor in cancerous ascites fluid elicited fatty acid release from rat epididymal adipose tissue in vitro. In this present study, it was attempted to find the inhibitory substance from red ginseng powder on lipolytic factor in ascites fluid of patients with hepatoma. Ginseng total saponin, protopanaxdiol and -triol saponin inhibited ACTH-induced lipolysis, but activated lipolysis induced by lipolytic factor. The inhibitory substance of lipolytic factor was found in saponin free extract of ginseng. The inhibitory substance significantly inhibited lipolysis induced by lipolytic factor, but did not exert any action on ACTH-induced lipolysis.

From the above results, it was suggested that administration of ginseng (saponin free extract) to cancer patients might improve anorexia and protect loss of body weight by inhibiting the actions of lipolytic factor secreted from tumor tissue.

INTRODUCTION

The depletion of lipid from the fat stores has been frequently observed in tumor-bearing animals and patients with various neoplastic diseases. This depletion could be related to growth of the tumor and could contribute to progressive weight loss on the host. However, the biochemical and physiological mechanism involved in this effect on the host has not been fully elucidated. Several possible mechanisms involved in this depletion of body fat are modifications of the rate of lipid deposition or of the rate of lipid mobilization. Earlier reports have shown that the incorporation rate of labelled glucose[1] and labelled acetate[10] into carcass lipids of tumor-bearing animals is much lower than those of normal rats. Frederick and Begg[2] early demonstrated that, when the tumors were removed surgically from tumor-bearing animals, the increased plasma free fatty acid levels resulted in return to normal values. Kralovic et al[6] reported that rats bearing the Walker 256 carcinoma have increased

plasma free fatty acid levels, and their epididymal adipose tissue has been shown to have basal lipolytic rate 2~3 times higher than those of tissue from normal rats. These observations raise the possibility that during the tumor growth, cancer cell produce a fat mobilizing factor which would stimulate lipolysis and/or affect the lipogenesis. Recently, Masuno et al[8] found a lipolytic substance in the cell-free ascites fluid of mice with Sarcoma 180 and patients with hepatoma or Grawitz[s tumor. They suggested that this substance "named Toxohormone-L" might be contribute to an increase in plasma free fatty acid and loss of body fat. Another investigation of this substance have shown that when, this substance was injected into the lateral ventricle of rats, food and water intake were significantly suppressed.[9]

Ginseng has been used widely as herbal medicine for many centries. The various biochemical and pharmacological effects of ginseng have been demonstrated. The antitumor effect of ginseng has not been fully accepted, but numerous datas have shown to antitumor activity of ginseng. Lee et al[7] reported that ether and ethanol extracts of ginseng inhibited the growth of Sarcoma 180 and Adenocarcinoma 755 transplanted mice. Hwang et al[4] demonstrated that a fat-soluble fraction of ginseng have cytotoxicity on leukemia cells. Recently, Yun et al[12] reported that administration of ginseng extracts in urethan treated mice resulted in an increased of natural killer activity. They suggested that anticarcinogenic effect of ginseng in urethan treated mice might be related to the augmentation of natural killer activity. However, active substance from ginseng on antitumor effect has not been clearly demonstrated. Therefore, it was attempted in the present study to find the active inhibitory substance from ginseng on lipolytic factor from cancerous ascites fluid.

MATERIALS AND METHODS

The following chemical reagents were purchased from Sigma chemical company : adrenocorticotropic hormone(ACTH), bovine serum albumin, 3-tertiary-butyl-4-hydroxyanisol(BHA), 2, 9-dimethyl-4, 7-diphenyl-1, 10-phenanthroline(bathocuproine). Whatman DEAE cellulose DE-52 was purchased from Whatman Ltd. All other chemical reagents were reagent grade commercially availible

Animals

Male Wister King rats, weighing 150~180g, were used. They were given standard diet and water ad libitum.

Preparation of ascites fluid

Ascites fluid were obtained from patients with hepatoma. The length of time between the death and obtaining the ascites fluid was 2 to 3 hours. The ascites fluid was centrifuged at 1000 g for 10 minutes at 4℃ to remove cell debris and the supernatant was stored at -20℃ until use. The lipolytic factor from cancerous ascites fluid was purified by procedure of Masuno et al.[8]

Estimation of lipolytic activity

Male rats were sacrified by a blow on the head and their epididymal adipose tissue was removed immediately. The adipose tissue were minced with scissors in Krebs-Ringer bicarbonate buffer(PH 7.4) and washed many times with same buffer. Then 100 mg of minced adipose tissue was incubated in a plastic test tube for 2 hours at 37℃ with 1 ml of Krebs-Ringer bicarbonate buffer(PH 7.4) containing 2.5% bovine serum albumin and 0.1 mM $CaCl_2$ in the prescence of various concentrations of ACTH and lipolytic factor. After incubation, 0.5 ml of reaction mixture without cell debris was added to the 6 ml of 1 : 1(V/V) mixture of chlor-

oform and n-heptane containing 2%(V/V) of methanol and extracted in a glass stoppered test tube by vigrously shaking for 10 minutes with shaker. The mixture was then centrifuged at 3000 rpm for 10 minutes and divided into two phases. The upper phase was discard by suction apparatus and added 2 ml of copper reagent The reaction mixture was vigrously shaked for 10 minutes and centrifuged at 3000 rpm. The 1 ml of upper organic phase, which contains copper salts of the extracted fatty acids, was reacted with 1 ml of color reagent(0.1% or bathocuproine and 0.05% of BHA(W/V) in chloroform). Colorimetric determination was performed at 480 nm. The value for released free fatty acid was corrected for the free fatty acid at 0 time. A control without ACTH and lipolytic factor was subjected to the same treatment. The net lipolytic activity induced by ACTH and lipolytic factor were calculated by substracting the free fatty acid content of the control.

One unit of lipolytic activity was defined at the amount causing release of 0.1 Eq free fatty acid in 2 hours.

Purification of saponin fractions and antilipolytic substance from red ginseng

Red ginseng powder was extracted 5 volumes of deionized water and the extract was concentrated under the vaccum pressure. The extract was treated with patroleum ether and chloroform successively to remove lipids. The saponin fraction from the extract was prepared by modified method of Han [3] The resultant extract without saponin fraction was dialyzed against deionized water and the inner dialysate was applied to active carbon column and eluted with 10%, 20% and 50% ethanol successively. The 50% ethanol fraction was applied to DEAE cellulose column equilibrated with 10 mM phosphate buffer(PH 8.0). The column was eluted with same buffer and then stepwise with phosphate buffer containing 0.2 M NaCl. The effluent from the column was collected in 10 ml fractions at a flow rate of 30 ml/hr. The summerized purification steps are shown in Fig. 1

RESULTS

Estimation of lipolysis induced by ACTH and lipolytic factor

The lipolytic activity in ACTH and ascites fluid of hepatoma patients were measured by incubation epididymal adipose tissue slices from normal male rats in Krebs-Ringer bicarbonate buffer(PH 7.4) containing various concentrations of ACTH and ascites fluid. The free fatty acid released from the adipose tissue slices were linearly dependent on the concentrations of added ACTH and ascites fluid(up to 10 μg and 0.2 ml) as shown in Fig. 2 and 3.

Effect of ginseng saponin fractions on ACTH and lipolytic factor

The effects of ginseng total saponin, protopanaxdiol and -triol saponin on ACTH induced lipolysis were measured by incubating epididymal adipose tissue slices in Krebs-Ringer bicarbonate buffer containing 0.1 μg of ACTH and 100 μg of total saponin, protopanax-dial and -triol saponin respectively. Total saponin did not affect on ACTH induced lipolysis, but protopanax-dial and -triol saponin inhibited 25% ACTH induced lipolysis(Fig 4).

The lipolytic activity of lipolytic factor in ascites fluid of hepatoma patients was activated 25% in the prescence of total saponin, protopanax-dial ,and -triol saponin(Fig. 5).

Effect of inhibitory substance on lipolytic factor

The non saponin extract was applied to active

carbon column and eluted with 10%, 20% and 50% ethanol successively. The inhibitory substance was found in 10% and 50% ethanol eluted fraction. The lipolytic activity of lipolytic factor was linearly inhibited by the concentrations of added inhibitory substance(up to 100 μg) as shown in Fig. 6, 7. However, these fractions did not affect on ACTH induced lipolysis.

The 50% ethanol eluated from the active carbon column was applied to DEAE cellulose column, previously equilibrated with 10mM phosphate buffer(Fig. 8). Solid line indicates the elution profile of carbohydrate estimated with phenol-sulfuric acid and dotted line shows the pattern of protein measured by the absorption at 280 nm. The most active inhibitory substance toward lipolytic factor was found in Fraction Ⅲ of non saponin fraction. The effect of Fraction Ⅲ toward lipolytic factor and ACTH-induced lipolysis are shown in Fig. 9. The inhibitory substance did not affect on ACTH-induced lipolysis, but selectively inhibited lipolysis induced by lipolytic factor.

DISCUSSION

During the progressive weight loss in various neoplastic diseases, depletion of fat stores on the host have been observed. The depletion of body fat during the growth of neoplasms in associated with increases in plasma free fatty acids One of the major substance involved in this effect is a lipolytic factor. The lipolytic substance produced by cancer cells increases the mobilization of free fatty acids from the adipose tissue. The existence of a lipolytic factor in tumor-bearing animals and various neoplastic diseases was supported by some evidences Kralovic et al[6] have shown that the ascites fluid of rats bearing the walker 256 carcinoma contains lipolytic substance that induces release of free fatty acids from the adipose tissue. Kitada et al[5] reported that the serum of mice with advanced lymphoma, tumor extracts and culture medium of lymphoma cells contains active lipolytic substance. Thompson et al[11] demonstrated that the endogeneous lipid synthesis and lipoprotein lipase activity in adipose tissue from tumor-bearing animals were significantly decreased and the basal rate of lipolysis was enhanced 2 hold in epididymal adipose tissue. These reports support the existence of lipolytic factor, but they did not isolate or identify the lipolytic factor. Masuno et al[8] purified and characterized lipolytic factor from cancerous ascites fluid as possible weight loss and anorexigenic factor. The purified lipolytic factor was acidic protein with an isoelectric point of 4.7 and molecular weight of about 70,000. They suggested that lipid depletion in tumor hosts may be explained in part by the action of lipolytic factor on the host.

In the present study, it was attempted to find inhibitory substance from ginseng powder toward lipolytic factor in cancerous ascites fluid.

The ginseng saponin fractions activated lipolysis induced by lipolytic factor and inhibited ACTH-induced lipolysis(Fig 4, 5.). The active inhibitory substance toward lipolytic factor was found in the extract of non saponin fraction. The non saponin fraction significantly inhibited lipolysis induced by lipolytic factor, but did not affect on ACTH-induced lipolysis(Fig. 6, 7 and 9.). These results suggest that inhibitory substance from ginseng powder do not exert any action on physiological hormone, but inhibit selectively lipolytic factor activity in cancerous ascites fluid. The active inhibitory substance might be a polysaccharide by estimation of phenol-sulfuric acid reaction and interesting that polysaccharide is a major component of ginseng.

From the above results, it seems likely that administration of ginseng(especially without saponin fraction) to cancer patients might improve

anorexia and protect loss of body weight by inhibiting the actions of lipolytic factor serected from tumor tissue.

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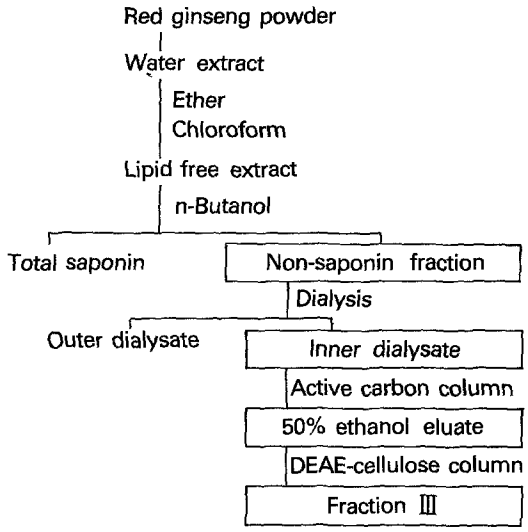

Fig. 1. Isolation of inhibitory substance toward lipolytic factor from red ginseng powder

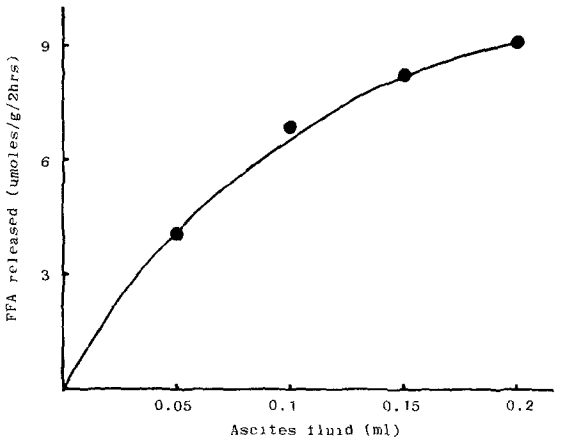

Fig. 3. Estimation of lipolytic activity induced by ascites fluid.

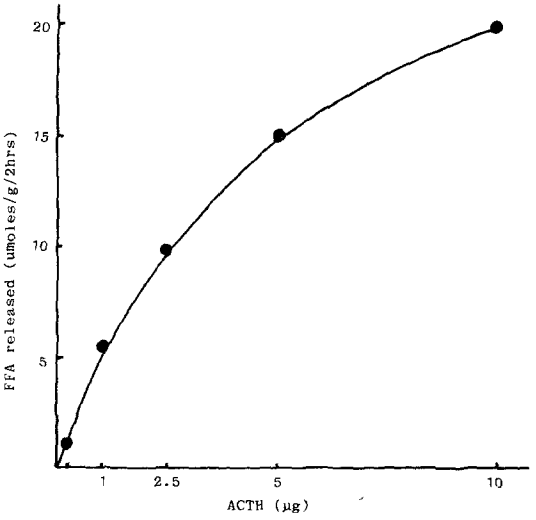

Fig. 2. Estimation of lipolysis induced by ACTH

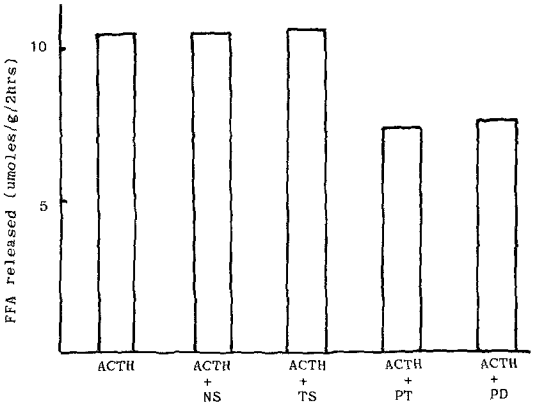

Fig. 4. Effect of ginseng fraction on ACTH-induced lipolysis.

NS : Non-saponin fraction
TS : Total saponin
PD : Protopanaxadiol saponin
PT : Protopanaxatriol saponin

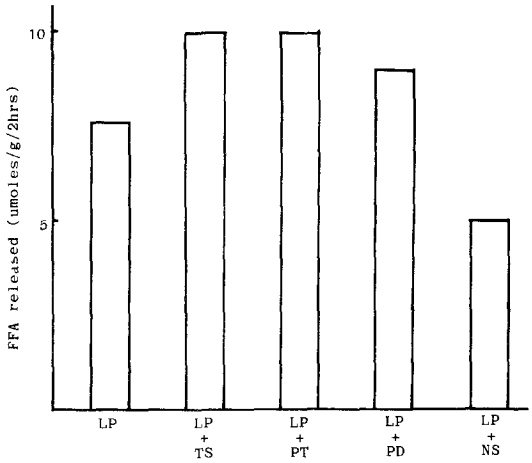

Fig. 5. Effect of ginseng fractions on lipolytic factor-induced lipolysis.
LP : Lipolytic factor
NS : Non-saponin fraction
TS : Total saponin
PD : Protopanaxadiol saponin
PT : Protopanaxatriol saponin

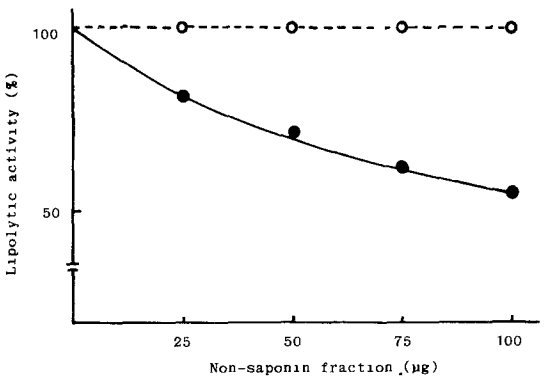

Fig. 7. Effect of 50% ethanol fraction of non-saponin extract on ACTH and lipolytic factor induced lipolysis.
○···○ : ACTH-induced lipolysis
●—● : Lipolytic factor-induced lipolysis

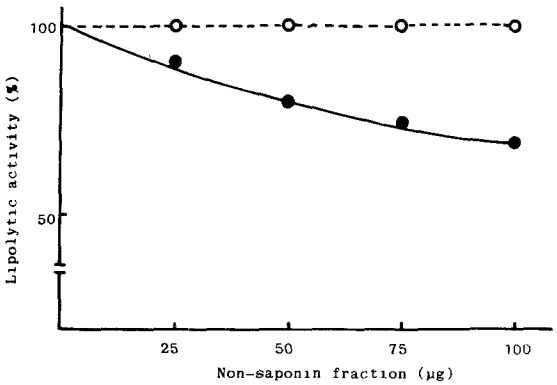

Fig. 6. Effect of 10% ethanol fraction of non-saponin extract on ACTH and lipolytic factor induced lipolysis.
○···○ : ACTH-induced lipolysis
●—● : Lipolytic factor-induced lipolysis

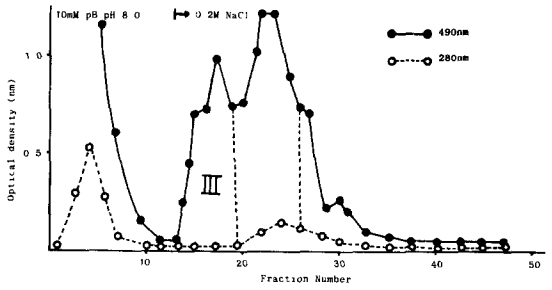

Fig. 8. DEAE-cellulose column chromatography of 50% ethanol eluate from active carbon column.

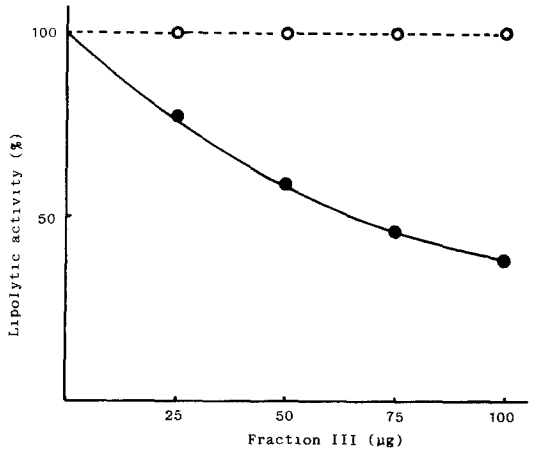

Fig. 9. Effect of fraction Ⅲ on ACTH and lipolytic factor induced lipolysis.
○…○ : ACTH-induced lipolysis
● — ● : Lipolytic factor-induced lipolysis