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Inhibitory Activity of Korean Ginseng on Lipolytic Action of Toxohormone-L from Cancerous Ascites Fluid

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Abstract

A substance that inhibit the lipolytic action of Toxohormone-L was a crude acidic polysaccharide isolated from Korean red and white ginseng. The total inhibitory activities(units) of PG_1 and PG_4 fraction in white ginseng and those of PG_1, PG_4 and $PG_{4\cdot3}$ fraction in red ginseng were higher than other fractions *in vitro* test.

Each water extract of ginseng was effective against the lipolysis induced by the Toxohormone-L at the concentration over 10~100 μl /ml. The total inhibitory activities (units) were highest at the concentration of 100 μg /ml and 1,000 μg /ml in the 4-year and 5-year old white ginseng root respectively, while, it was higher in the 6-year old ginseng than other ages regardless of the reaction concentration in the red ginseng.

The inhibitory effect of ginsenoside $-Rb_2$ on the lipolysis by Toxohormone-L was higher than other ginsenosides at the concentration of 100 μg to 500 μg /ml of reaction mixture, and total inhibitory activities (units) of ginsenoside $-Rb_2$ were also higher than other treatments.

Key words : Korean ginseng, toxohormone-L

INTRODUCTION

Ginseng has been believed by most of the orientals for thousands of years, to cure all diseases as indicated in its scientific name, *Panax ginseng* C. A. Meyer[1, 2]. It has proved its efficacy and maintained its supremacy as a king of the medicinal herbs. Many scientists have been studying on various aspects of the plant such as efficacy[3], pharmacology[4], and components[5] and they try to unveil the hidden characters of the mysterious herbs.

There are many experimental evidence to demonstrate the antitumor action in the various cancer cells[6, 7] and animal[8~10].

During the last 20 years, panax ginseng has become recognized in its efficacy as a supplementary treatment medicine, as well as an inhibitor of cancer[11, 12].

Depletion of fat stores has been observed in patients with various neoplastic diseases and tumor-bearing animals during progressive weight loss and these findings during growth of neoplasms are associated with increase in the plasma level of free fatty acids[13]. Although the physiological mechanisms by which cancer leads to depletion of body lipids is unknown, it could be due to cancer-mediated stimulation of free fatty acids release from the host's adipose tissues.

Previously, Okuda etc found a lipolytic factor in the ascites fluids obtained from mice with

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sarcoma 180 and patients with hepatoma. So this factor may contribute to increase in plasma free fatty acids and decrease in body lipids. A lipolytic factor, named "Toxohormone-L", was purified from the ascites fluid of sarcoma 180-bearing mice[14].

Injection of Toxohormone-L into the lateral ventricle of rats significantly suppressed their food and water intakes. Therefore, Toxohormone-L has two actions, lipolytic and anorexigenic actions, which may cause reduction of body fat in cancer patients.

In the present investigation[15], we tried to measure the inhibitory activity in Korean ginseng powder that inhibit the lipolytic action of Toxohormone-L in ascites fluid of sarcoma 180-bearing mice.

This study was devised to isolate the acidic polysaccharide from Korean red and white ginseng powder that inhibit the lipolysis induced by Toxohormone-L in ascitic fluid of sarcoma 180-bearing mice and compare the inhibitory effect of the acidic polysaccharide fraction and water extract of white & red ginseng and ginsenoside.

MATERIALS AND METHODS

1. Animals

Young male and Wistar King rats and Sprague-Dawley rats, weighing 170 to 200 g, were allowed free access to standard laboratory diet and water. They were sacrificed by a blow on the head and their epididymal adipose tissues were quickly removed. Male DDK and Swiss mice, weighing 17 to 20 g, were also given standard laboratory diet and water and *libitum*.

2. Red ginseng and white ginseng

Red ginseng powder (*Panax ginseng* C. A. Meyer) was kindly provided by Nikkan Korai Ninjin Co. Ltd., Kobe, Japan and Korea Ginseng and Tobacco Research Institute, Deajeon, Korea.

3. Ginsenoside and water extract ginseng

Ginsenoside of red ginseng and water extract of Korean white and red ginseng was kindly provided by Korean Ginseng and Tobacco Research Institute, Deajeon, Korea.

4. Preparation of Toxohormone-L fraction

Male DDK mice were inoculated i. p. with 0.5 ml of sarcoma 180 suspension (4 to 5 $\times 10^9$ cells / mouse), and 10 to 14 days later, the ascites fluid was harvested. The ascites fluid was centrifuged at 1,000 × g for 10 min. at 4℃ and the resultant supernatant was used as the Toxohormone-L fraction.

5. Measurement of anti-lipolytic activity

Isolated fat cells were prepared from rat epididymal adipose tissue by the method of Rodbell1[6]. Fat cells (50 μl packed volume) were incubated for 30 min. at 37℃ in 175 μl of Hanks buffer (pH 7.4) containing 25 mM HEPES, 4% bovine serum albumin, and 25 μl of the test sample and 50 μl of Toxohormone-L fraction in a final volume of 0.30 ml. After incubation, the free fatty acids released were extracted with 3 ml of a 1:1 (v/v) mixture of chloroform and heptane containing 2%(v/v) methanol and measured with copper reagent and bathocuproine by the method of Zapf *et al*[17].

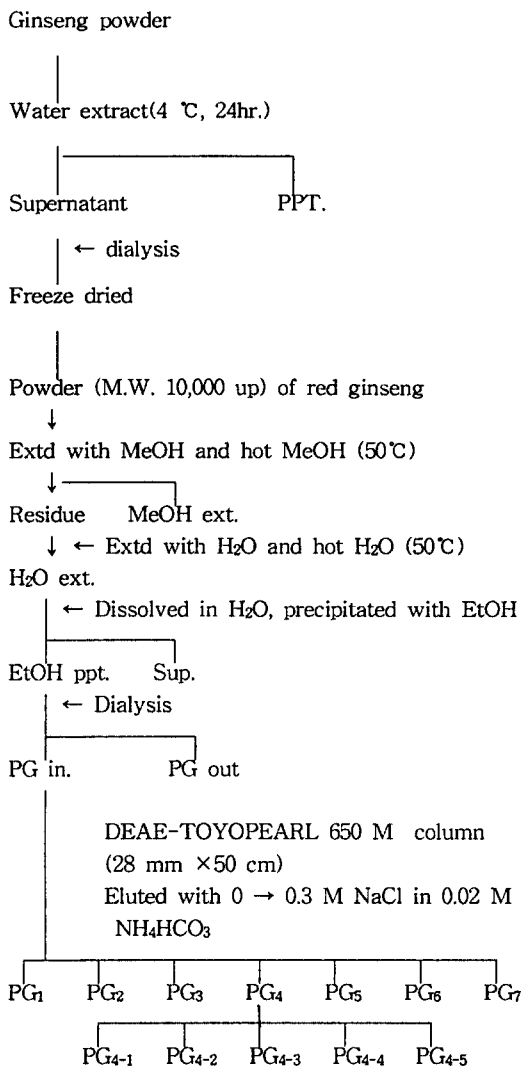
6. Purification of acidic polysaccharide

Red ginseng and white ginseng powder was extracted with 10 volumes of deionized water at 4℃ for 24 hr. The extract was centrifuged, and the supernatant was concentrated and dialyzed against deionized water at 4℃ for 24 hr. to re-

move the molecules smaller than 10,000 daltons. The inner dialysate was then concentrated and freeze-dried. The resulting powder was treated with methanol at room temperature and then with hot methanol (50℃) to remove ginsenosides. The residual material was extracted with deionized water at room temperature and then with hot water (50℃), and then the water extracts were combined, concentrated and mixed with 4 volumes of ethanol. The resulting precipitate fraction (ginsenoside-free ethanol precipitate) was dialyzed against deionized water and the inner dialysate was applied to a DEAE-TOYOPEARL 650 M column (28 mm × 50 cm) equilibrated with 0.02 M NH_4HCO_3. Elution was carried out with 0 M, 0.05 M, 0.10 M, 0.15 M, 0.20 M, 0.25 M and 0.30 M NaCl in 0.02 M NH_4HCO_3, successively. The resulting fractions of eluate were named PG_1, PG_2, PG_3, PG_4, PG_5, PG_6 and PG_7, respectively. To purify the PG_4 fraction, the gradient elution was carried out with the same column from 0 to 0.3 M NaCl in 0.02 M NH_4HCO_3. The resulting fractions of eluates were named $PG_{4\text{-}1}$, $PG_{4\text{-}2}$, $PG_{4\text{-}3}$, $PG_{4\text{-}4}$, $PG_{4\text{-}5}$, respectively.

RESULTS AND DISCUSSION

Ginseng has capacity for enabling living organism to adap to various adverse action coming from environmental circumstances. Besides, ginseng has antifatigue action, antistress effect, hypotensive effects, anticancer action, and so on[18]. Especially, Hwang[19] found that petroleum ether extract of ginseng has anticancer effect and Kim[20] clarified the structure formula of the active component. Also, Lee etc.[21, 22] found a acidic polysaccharide fractions from red and white ginseng to inhibit the lipolysis induced by Toxohormone-L.



PG_1 : 0.02 M NH_4HCO_3 aq. fr.
PG_2 : 0.05 M NaCl/0.02 M NH_4HCO_3 aq. fr.
PG_3 : 0.10 M NaCl/0.02 M NH_4HCO_3 aq. fr.
PG_4 : 0.15 M NaCl/0.02 M NH_4HCO_3 aq. fr.
PG_5 : 0.20 M NaCl/0.02 M NH_4HCO_3 aq. fr.
PG_6 : 0.25 M NaCl/0.02 M NH_4HCO_3 aq. fr.
PG_7 : 0.30 M NaCl/0.02 M NH_4HCO_3 aq. fr.

Fig. 1. Schemativ flow diagram describing the major step of acidic polysaccharide isolation.

We isolated the acidic polysaccharide fractions from Korean red and white ginseng. The yields of the acidic polysaccharide fractions(PG_1-PG_7) from 500 g of red ginseng powder were 32.3 g, 777 mg, 311 mg, 197 mg, 94 mg, 25 mg and 25 mg, respectively. And the yields of these fractions from 500 g of white ginseng powder were 4.28 g, 35 mg, 5 mg, 160 mg, 85 mg, 15 mg and 30 mg, respectively(Table 1). So the yields of the red ginseng were higher than those of the white ginseng.

The inhibitory effects of the acidic polysaccharide fractions on lipolysis induced by Toxohormone-L were examined. As shown in Table 2, PG_1, the unabsorbed fraction, and PG_4 have strong inhibitory effects. And inhibitory effects of purified fractions from PG_4 were examined. As shown in Table 3, inhibitory of effect $PG_{4\text{-}3}$ was highest of all the treatment at all concentration except 10 μg/ml.

To consider both the inhibitory effects and the yields of PG fractions, the total inhibitory activities(units/g) of the acidic polysaccharide fraction from red ginseng on lipolysis induced by Toxohormone-L were examined.

1 unit[23] were acquired from 10% of total inhibitory ratio per g of ginseng sample. And total inhibitory ratio was obtained by calculating (the inhibition ratio per μg of PG fraction × total weight of PG fraction). Therefore, total inhibitory activities offer a good information for the selection of the ginseng sample or the comparison of the experimental data.

As shown in Table 4, total inhibitory activity of PG_4 was higher than other fractions except unabsorbed fraction(PG_1). And total inhibitory activity of $PG_{4\text{-}3}$ on lipolysis was highest of all the fraction of PG_4 of red ginseng(Table 5).

Table 1. Yield of acidic polysaccharide acquined from red and white ginseng 500g

Species	Fraction						
	PG_1	PG_2	PG_3	PG_4	PG_5	PG_6	PG_7
	mg						
Red ginseng	32,300	777	311	197	94	25	25
White ginseng	4,280	35	5	160	85	15	30

Table 2. Inhibitory effects of polysaccharide fraction (PG) fraction from red ginseng on lipolysis induced by Toxohormone-L

Concentration	Fraction						
(μg/ml)	PG_1	PG_2	PG_3	PG_4	PG_5	PG_6	PG_7
	Percent inhibition						
10	12.2	−1.1	−2.5	−1.7	−1.5	−4.9	−6.6
50	35.8	3.3	9.0	22.0	4.2	13.6	18.4
100	44.4	10.9	24.5	42.7	27.1	25.1	25.0
200	47.3	11.6	25.9	53.2	32.0	28.1	25.9
500	62.3	12.5	35.1	72.2	52.9	42.0	27.4
1,000	80.0	19.9	45.0	87.9	61.1	31.9	–

The rate of Toxohormone-L-induced lipolysis was 2.23 free fatty acid μEq/g cells/2 hrs in the absence of PG fraction.

Table 3. Inhibitory effects of various fractions obtained by gradient elution on lipolysis induced by Toxohormone-L

Concentration	Fraction				
(μg /ml)	$PG_{4\text{-}1}$	$PG_{4\text{-}2}$	$PG_{4\text{-}3}$	$PG_{4\text{-}4}$	$PG_{4\text{-}5}$
	Percent inhibition				
10	13.1	20.3	11.3	26.7	−1.5
50	14.8	31.6	66.7	44.8	7.6
100	40.2	47.6	80.2	65.1	19.6
200	40.6	48.4	82.5	70.5	20.0
500	76.6	52.2	97.7	88.4	76.1
1,000	79.1	59.9	98.9	91.3	–

The rate of Toxohormone-L-induced lipolysis was 2.46 free fatty acid μEq /g cells /2hrs in the absence of PG fraction.

Table 4. Total inhibitory activity of the acidic polysaccharide fraction(PG) from red ginseng on Toxohormone-L induced lipolysis

Concentration	Fraction						
(μg /ml)	PG_1	PG_2	PG_3	PG_4	PG_5	PG_6	PG_7
	Unit* / g						
10	7,881	−17	−16	−7	−3	−3	−3
50	4,625	10	11	17	2	1	2
100	2,868	17	15	17	5	1	1
200	1,528	9	8	10	3	1	1
500	805	4	4	6	2	0	0
1,000	517	3	3	3	2	0	0

unit = 10% inhibition /g of red ginseng.

Table 5. Total inhibitory activity of the acidic polysaccharide fractions from PG_4 fraction of red ginseng on Toxohormone-L induced lipolysis

Concentration	Fraction				
(μg /ml)	$PG_{4\text{-}1}$	$PG_{4\text{-}2}$	$PG_{4\text{-}3}$	$PG_{4\text{-}4}$	$PG_{4\text{-}5}$
	Unit* / g				
10	1	2	22	31	0
50	0	1	25	10	0
100	0	1	15	8	0
200	0	0	8	4	0
500	0	0	4	2	0
1,000	0	0	2	1	0

* All fractions obtained by gradient elution from PG_4 fraction of acidic polysaccharide.

*1 unit = 10% inhibition /g of red ginseng.

The inhibitory effects of the acidic polysaccharide fractions of white ginseng on lipolysis induced by Toxohormone-L were examined. As shown in Table 6, PG_1, the unabsorbed fraction, and PG_5 had strong inhibitory effect. But total inhibitory activities(units) of PG_1 and PG_4 were higher than other treatment at all concentration(Table 7).

As shown in Table 2, 3 & 6, the inhibitory effects of the acidic polysaccharide fractions of red and white ginseng on lipolysis were increased according to the increase of the concentration. But the total inhibitory activities (unit /g) of PG fractions of red and white ginseng were decreased as concentration. These results were due to the fact that the inhibitory effects on lipolysis were not proportional to the concentration of PG fractions.

The effects of water extracts from 4-, 5- and 6-year old red and white ginseng roots on the inhibition of lipolysis in fat cells induced by Toxohormone-L were examined. The inhibition ratio of lipolysis by the water extract of 4-, 5- and 6-year old white ginseng roots were 56.3, 59.7, and 59.4%, respectively at 1,000 μg /ml concentration. And those of red ginseng roots were 78.6, 79.1 and 82.5%, respectively, indicating that the 6-year old red ginseng was the most effec-

Table 6. Inhibitory effects of the acidic polysaccharide fraction from white ginseng on lipolysis induced by Toxohormone-L

Concentration	Fraction						
(μg /ml)	PG_1	PG_2	PG_3	PG_4	PG_5	PG_6	PG_7
	Percent inhibition						
10	19.4	18.3	8.7	23.3	7.4	22.7	29.5
50	26.2	26.7	21.3	33.0	44.6	32.6	32.4
100	37.3	31.1	21.7	44.8	57.7	45.3	34.4
200	36.2	29.9	23.2	54.0	73.5	47.7	42.6
500	35.8	31.9	34.3	62.7	85.4	80.0	49.9
1,000	36.2	67.4	70.3	100	100	97.1	100

The rate of Toxohormone-L induced lipolysis was 2.01 free fatty acid μEq /g cells /2 hrs in the absence of PG fractions.

Table 7. Total inhibitory activity of the acidic polysaccharide fraction from white ginseng on Toxohormone-L induced lipolysis

Concentration	Fraction						
(μg /ml)	PG_1	PG_2	PG_3	PG_4	PG_5	PG_6	PG_7
	Unit* / g						
10	1,661	13	1	75	13	7	18
50	449	4	0	21	15	2	4
100	319	2	0	14	10	1	2
200	155	1	0	9	6	0	1
500	61	0	0	4	3	0	0
1,000	31	0	0	3	2	0	0

*1 unit = 10% inhibition /g of white ginseng.

tive in the inhibition of the lipolysis(Table 8).

The total inhibitory activities per gram of ginseng sample were high in the 4-year and 5-year old white ginseng roots at the concentrations of 100 μg/ml and 1,000 μg/ml, respectively, while for the red ginseng it was high in the 6-year old ginseng at the concentration of 100~1,000 μg/ml(Table 9).

Finally, *in vitro* test showed that the inhibitory effect of -Rb_2 on the lipolysis by Toxohormone-L was highest of all the ginsenoside treatments (Table 10). And total inhibitory activity(units) of -Rb_2 was also highest of all the treatments at all concentration except 10 μg/ml

Table 8. Inhibitory effect of water extract of Korean white and red ginseng on lipolysis* induced by Toxohormone-L

Root age(year) / Conc.(μg/ml)	White ginseng			Red ginseng		
	4	5	6	4	5	6
	% inhibition					
10	1.4	−1.4	4.4	5.4	4.7	5.0
100	12.7	12.9	13.0	14.3	14.0	16.3
1,000	56.3	59.7	59.4	78.6	79.1	82.5
10,000	92.9	92.8	94.3	94.8	96.1	98.6

* The rate of Toxohormone-L induced lipolysis was 0.38 free fatty acid Eq/g cells/2hrs in the absence of water extract of Korean ginseng.

Table 9. Total inhibitory activity of water extract of Korean white and red ginseng on lipolysis induced by Toxohormone-L

Root age (year) / Conc.(μg/ml)	White ginseng			Red ginseng		
	4	5	6	4	5	6
	Unit* / g					
10	4,424	−4,298	13,112	21,816	19,458	19,900
100	4,013	3,960	3,874	5,777	5,798	6,487
1,000	1,779	1,833	1,770	3,175	3,275	3,284
10,000	294	285	281	383	398	392

* 1 unit = 10% inhibition / g of ginseng.

Table 10. Inhibitory effect of ginsenosides on lipolysis* induced by Toxohormone-L

Concentration (μg/ml)	Ginsenosides						
	Rb_1	Rb_2	Rc	Rd	Re	Rg_1	Rg_2
	% inhibition						
10	−2.2	1.6	4.9	−3.6	0.0	2.6	4.3
100	1.1	23.0	13.1	5.4	5.7	8.6	8.6
500	5.0	56.7	28.3	8.3	15.5	15.7	14.9
1,000	13.0	63.0	32.6	34.8	26.9	24.5	34.2

* The rate of Toxohormone-L induced lipolysis was 0.52 free fatty acid μEq/g cells/2hrs in the absence of ginsenoside.

Table 11. Total inhibitory activity of the ginsenoside fraction from Korean red ginseng on Toxohormone-L induced lipolysis

Concentration	Ginsenosides						
(μg /ml)	Rb_1	Rb_2	Rc	Rd	Re	Rg_1	Rg_2
				Unit* / g			
10	−75	37	152	29	0	68	17
100	4	53	41	4	36	22	3
500	3	26	18	1	20	8	1
1,000	4	15	10	3	17	6	1

* 1 unit = 10% inhibition / g of ginseng.

(Table 11).

Up to the present, it is of common knowledge that the pharmacological effects of Korean ginseng are caused by the ginsenosides. According to the result of this study, the acidic polysaccharide fractions had the inhibitory effects on lipolysis induced by Toxohormone-L as ginsenosides. And the inhibitory effects on lipolysis were stronger in PG fractions than on ginsenosides. But the total inhibitory activities of ginsenosides on lipolysis were higher than those of PG fractions.

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高麗人蔘이 癌毒素의 脂肪分解作用에 미치는 沮害活性

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요 약

한국 홍삼과 백삼의 조산성 다당체 성분과 수용성 추출물, 그리고 ginsenoside 성분이 암독소 호르몬-L 에 의해 유도된 지방 분해를 저해하는 효과를 검토하고자 본 연구를 수행하였다. 지방분해 및 식욕억제 인자로 알려진 암독소 호르몬-L은 sarcoma-180을 접종한 mouse의 복수액으로 부터 부분정제하여 사용하였다. 한국 홍삼과 백삼으로부터 정제하여 얻은 조산성 다당체 각 분획의 성분은 10 μg /ml 농도 이상에서 암독소 호르몬-L 이 유도하는 지방 분해에 억제효과가 있었고, 이들 중 PG_1 과 PG_4 에서 지방 분해 저해 활성이 가장 높았으며, 이를 더 정제한 결과 $PG_{4\text{-}3}$ 분획에서 활성이 가장 높았다. 고려인삼 중 백삼과 홍삼의 각 4, 5, 6 년근별로 얻은 수용성 추출물의 암독소 호르몬-L의 지방분해 억제에 미치는 영향을 검토한 결과 억제율은 1,000 μg /ml 농도에서 백삼 4, 5 및 6년근이 각각 56.3, 59.7 및 59.4% 였고, 홍삼은 각각 78.6, 79.1 및 82.5%로 백삼보다 홍삼의 억제율이 높았다. 인삼 시료 g 당 총저해활성(unit /g)은 백삼의 경우 100 μg /ml 농도에서는 4년근이, 1,000 μg /ml 농도에서는 5년근에서 가장 높았으나, 홍삼의 경우 다같이 6년근에서 가장 높았다. 또한 ginsenoside 성분의 경우 지방 분해 억제율과 총저해활성이 모두 Rb_2에서 가장 높았다.