

Anticarcinogenic Effect of Ginseng Extracts Depending on the Types and Ages Using Yun's Anticarcinogenicity Test(II)

Taik-Koo Yun* and Yun-Sil Lee¹

Laboratory of Cancer Pathology and ¹Laboratory of Epidemiology,
Korea Cancer Center Hospital, Seoul 139-240, Korea

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Abstract□ In this study, we investigated the anticarcinogenicity of various types and ages of ginseng extracts as an extended study using Yun's anticarcinogenicity test. Fresh ginseng at 1.5, 3, 4, 5 and 6 years was dried and powdered. And white ginseng was processed in the same way that of fresh ginseng after removal of the ginseng cortex and fine root. For red ginseng, fresh ginseng was steamed and dried. Each ginseng powder was extracted and extracts was freeze dried. Newborn N : GP(S) mice were given a single subcutaneous injection of 0.5 mg of benzo(a)pyrene(BP). Various types and ages of ginseng extracts at 2.5 mg/ml were orally administered. All the mice were sacrificed at the 9th week. The following results were obtained. In the dried fresh ginseng extract treated group, the incidence of lung adenoma induced by BP was 63.9% and its incidence was reduced to 48.3%, 52.5%, 51.8%, 47.5% and 44.1% after co-treatment with 1.5, 3, 4, 5 and 6 year-dried fresh ginseng, respectively. The incidence of lung adenoma induced by BP on the white ginseng extract treated group was 41.3% and decreased to 31.0%, 46.0%, 44.0% and 26.5% after co-treatment with 3, 4, 5 and 6 year-white ginseng, respectively. In the red ginseng extract treated group, the incidence of lung adenoma induced by BP was 47.5% and its incidence diminished to 40.7%, 35.0%, 30.1%, 30.0% and 26.3% after co-treatment with 1.5, 3, 4, 5 and 6 year-red ginseng, respectively. From the above results, we concluded that a statistically significant anticarcinogenic effect was observed in extracts of 6 year-dried fresh ginseng, 6 year-white ginseng, and 4, 5 and 6 year-red ginseng and it is suggested that the anticarcinogenicity of ginseng varies according to the types and ages.

Key words□ Ginseng extract, types and ages, anticarcinogenic, newborn mice, lung tumor.

Introduction

Panax ginseng has been extensively used in traditional oriental medicine as a restorative tonic and prophylactic agent.¹⁾ The pharmacological effects of ginseng and its extract reported during the past several decades are various and controversial. The primary pharmacological activities of ginseng are the central nervous system, gonadotropism, antistress, RNA synthesis, DNA and lipid and protein synthesis.^{2,3)} Several investigators have demonstrated the anticancer effect of ginseng in various transplantable tumor models using rodents.^{4,5)}

The authors have already shown that 6-year-old red ginseng extract had remarkable anticarcinogenic effects on lung tumors induced by dimethylbenz (a)anthracene, urethan, and aflatoxin B₁ in long term carcinogenesis models^{6,7)} and preventive effects of ginseng on cancer.^{8,9)} The anticarcinogenicity of red ginseng extract was also shown in nine-week medium term bioassay model induced by benzo(a)pyrene.^{10,11)} We have also shown that ginseng powders had an anticarcinogenic effect in mice and that their effects varied according to the types and ages of ginseng used.¹²⁾

Therefore, in this study, we further investigated whether the extract of fresh ginseng or white ginseng had similar anticarcinogenic properties compa-

*To whom correspondence should be addressed.
Tel : (02) 977-0381.

red with ginseng powders, and if their anticarcinogenicity would also relate to the types and ages of ginseng, using Yun's anticarcinogenicity test.

Materials and Methods

1. Experimental animals

Non-inbred N:GP(S) mice were obtained from the NCI (National Cancer Institute, U.S.A. [NIH]) and bred at random *inter se*. All mice were housed in a controlled room, fed solid pellets prescribed by NIH-7-open formula and given water *ad libitum*.

2. Preparation of ginseng extracts

Fresh ginseng at 1.5, 3, 4, 5 and 6 years was processed in three ways and extracted in a water bath for 8 hours, a total of three times and the extracts were freeze dried (yield: 45%, 47% and

51% in fresh ginseng, white ginseng and red ginseng, respectively).

- ① Fresh ginseng was dried at room temperature and finely powdered and extracted in a water bath for 8 hours, 3 times for dried fresh ginseng.
- ② White ginseng was processed in the same way as fresh ginseng after removal of the ginseng cortex and fine root.
- ③ For red ginseng, fresh ginseng was steamed and dried.

3. Treatment

Newborn mice, less than 24 hours old, were injected subcutaneously in the scapular region with 0.02 ml of the suspension, containing 0.5 mg of benzo(a)pyrene (BP, Sigma Chemical Co., USA) in 1% aqueous gelatin. After weaning, various ages of dried

Table 1. Body and lung weights

Experimental group	Sex	Ginseng extract					
		Fresh ginseng		White ginseng		Red ginseng	
		Body wt*	Lung wt**	Body wt	Lung wt	Body wt	Lung wt
Untreated	M	32.7±2.8	7.3±1.1	35.5±2.3	7.2±0.7	31.5±2.4	8.8±1.4
Control	F	27.1±3.1	8.8±0.5	29.1±3.3	8.6±2.3	26.4±1.7	9.8±1.2
BP	M	31.1±2.7	7.1±1.0	34.3±2.7	6.8±0.6	30.3±3.1	8.6±1.7
	F	27.1±2.2	8.3±1.0	26.8±2.6	8.7±1.1	25.7±2.7	9.7±1.8
1.5 year***	M	32.3±3.2	8.3±1.7	—	—	34.1±1.8	8.1±1.3
	F	27.9±2.7	8.3±0.7	—	—	26.4±2.4	9.6±2.4
BP+1.5 year	M	33.1±2.5	8.3±1.1	—	—	32.2±3.5	9.6±1.5
	F	28.4±2.4	9.9±0.8	—	—	26.1±2.8	9.1±1.5
3 year	M	32.1±2.2	7.2±0.7	35.5±2.7	7.6±1.1	33.5±2.1	7.9±9.8
	F	26.1±2.3	7.9±1.0	27.9±2.7	8.9±1.1	25.3±2.1	11.1±2.8
BP+3 year	M	34.1±2.1	8.4±1.6	34.2±2.4	8.6±1.0	32.1±2.8	8.7±1.5
	F	27.5±1.6	10.9±1.8	25.6±2.5	10.8±2.1	26.1±2.2	11.6±1.1
4 year	M	31.4±3.6	7.4±0.8	35.1±3.4	7.7±0.9	33.5±2.1	7.9±1.3
	F	26.8±1.8	8.7±0.7	26.1±2.3	8.4±1.0	25.3±1.9	7.7±1.3
BP+4 year	M	32.7±2.6	9.1±1.2	33.9±2.7	7.2±0.6	32.0±2.6	7.9±0.6
	F	27.2±2.8	9.3±1.2	27.5±2.6	8.2±1.0	25.2±2.2	8.6±1.2
5 year	M	31.9±2.3	8.2±1.0	34.5±2.2	7.5±0.5	32.6±2.7	9.2±1.4
	F	26.2±2.8	10.5±1.3	27.0±2.3	8.9±1.2	25.7±2.2	9.0±1.6
BP+5 year	M	32.7±1.7	8.6±1.0	33.8±2.9	7.3±0.8	32.3±3.5	7.3±1.2
	F	26.4±2.7	9.0±1.2	26.5±2.8	8.0±1.0	25.8±2.7	9.6±1.5
6 year	M	32.3±2.4	9.5±0.9	35.1±2.6	7.5±0.9	30.9±2.1	9.6±1.5
	F	26.6±1.9	9.3±1.5	27.9±1.9	9.5±1.6	25.4±1.9	9.1±1.5
BP+6 year	M	31.9±3.3	7.8±1.2	35.3±2.3	7.5±0.6	32.7±2.4	10.7±1.8
	F	26.8±2.6	9.9±1.2	27.9±3.0	8.5±1.0	26.6±1.9	11.2±1.3

Data represents Mean±S.D., BP: Benzo(a)pyrene.

*Gram, **mg/g body weight, ***Age of ginsengs.

fresh ginseng, white ginseng and red ginseng extracts at a concentration of 2.5 mg/ml were orally administered in the drinking water for 6 weeks after weaning. All the mice were sacrificed at the 9th week.

4. Bioassay of anticarcinogenicity

The present experiment adopted the 9 week medium-term bioassay model established in the author's laboratory, and called 'Yun's anticarcinogenicity test' in our laboratory to distinguish this test from other medium-term bioassay models.

5. Scoring of lung tumors

To determine the number of tumors per lung, the animals were killed by asphyxiation. The lung were excised, fixed in Tellyesniczky's solution, and the incidence and multiplicity of lung adenoma were counted with the naked eye.

6. Statistical analysis

Chi-square test was used for tumor incidence and Student's t-test for multiplicity, organ and body weight were used.

Results

Table 1 shows that the animals were tolerated carcinogen or carcinogen combined with various types of ginseng extract treatment. There was no mortality attributable to the treatment, and overall weight gain over the 9 week period was almost the same between control and treated animals. Mean relative lung weight of each group did not show any differences between the groups. In the dried fresh ginseng extract treated group, the incidence of lung adenoma induced by BP was 63.9% and its incidence was reduced to 48.3%, 52.5%, 51.8%, 47.5% and 44.1% after co-treatment with 1.5, 3, 4, 5 and 6 year-dried fresh ginseng extracts, respectively. A significant effect was observed only in 6 year-dried fresh ginseng extract ($p < 0.05$) (Table 2). In the white ginseng extract treated group, the incidence of lung adenoma induced by BP was 41.3% and its incidence decreased to 32.0%, 46.0%, 44.0% and 26.5% after co-treatment with 3, 4, 5 and 6 year-white ginseng extracts, respectively. Six year-ginseng extract showed significant inhibition of lung adenoma ($p < 0.05$) (Table 3). In the red gin-

Table 2. Effect of fresh ginseng extract on the incidence of lung adenoma in mice treated with benzo(a)pyrene

Group and treatment	Mice		Incidence (%)	Multiplicity (mean \pm S.D)
	Sex	Number		
Untreated control	M	30	0	0
	F	30	0	0
	M+F	60	0	0
Benzo(a)pyrene (BP, 0.5 mg)*	M	26	57.7	1.76 \pm 2.06
	F	30	70.0	1.30 \pm 1.26
	M+F	56	63.9	1.78 \pm 2.12
1.5 years** (5 mg/ml)***	M	30	0	0
	F	29	0	0
	M+F	59	0	0
BP+1.5 years	M	30	46.7	1.77 \pm 3.01
	F	29	50.0	1.47 \pm 2.71
	M+F	59	48.3	1.61 \pm 2.84
3 years	M	30	0	0.03 \pm 0.12
	F	30	4.0	0.04 \pm 0.28
	M+F	60	2.0	0.02 \pm 0.14
BP+3 years	M	30	53.3	1.27 \pm 1.63
	F	29	51.7	1.72 \pm 3.66
	M+F	59	52.5	1.59 \pm 2.86
4 years	M	30	0	0
	F	30	0	0
	M+F	60	0	0
BP+4 years	M	30	50.0	1.13 \pm 1.89
	F	29	53.6	1.14 \pm 1.38
	M+F	59	51.8	1.13 \pm 1.65
5 years	M	30	0	0
	F	30	4.0	0.04 \pm 0.28
	M+F	60	2.0	0.02 \pm 0.14
BP+5 years	M	30	46.7	1.70 \pm 4.27
	F	29	48.3	2.00 \pm 2.66
	M+F	59	47.5	1.79 \pm 3.48
6 years	M	30	0	0
	F	30	0	0
	M+F	60	0	0
BP+6 years	M	27	48.3	1.02 \pm 1.58
	F	30	40.0	1.30 \pm 2.07
	M+F	57	44.1 ¹	1.16 \pm 1.72

BP : Benzo(a)pyrene.

*Per mouse, subcutaneous injection.

** Age of fresh ginseng.

***ml in drinking water for 6 weeks.

Significantly different from BP alone group at ¹ $p < 0.05$.

seng extract treated group, the incidence of lung adenoma induced by BP was 47.5% and its incidence diminished to 40.7%, 35.0%, 30.1%, 30.0% and 26.3% after co-treatment with 1.5, 3, 4, 5 and 6

Table 3. Effect of white ginseng extract on the incidence of lung adenoma in mice treated with benzo(a)pyrene

Group and treatment	Mice		Incidence (%)	Multiplicity (mean± S.D)
	Sex	Number		
Untreated control	M	25	0	0
	F	25	0	0
	M+F	50	0	0
Benzo(a)pyrene (BP, 0.5 mg)*	M	40	35.0	0.43± 0.68
	F	40	47.5	0.70± 1.37
	M+F	80	41.3	0.55± 0.83
3 years** (5 mg/ml)***	M	24	0	0
	F	25	4.0	0.04± 0.28
	M+F	49	2.0	0.02± 0.14
BP+3 years	M	25	28.0	0.32± 0.56
	F	25	36.0	0.72± 1.37
	M+F	50	32.0	0.52± 1.05
4 years	M	25	4.0	0.04± 0.28
	F	25	0	0
	M+F	50	2.0	0.02± 0.14
BP+4 years	M	25	36.0	0.64± 1.08
	F	25	56.0	1.16± 1.70
	M+F	50	46.0	0.90± 1.43
5 years	M	25	8.0	0.08± 0.20
	F	25	4.0	0.04± 0.28
	M+F	50	6.0	0.06± 0.24
BP+5 years	M	25	36.0	0.44± 0.65
	F	25	52.0	1.00± 1.35
	M+F	50	44.0	0.68± 1.08
6 years	M	25	4.0	0.04± 0.28
	F	25	4.0	0.04± 0.28
	M+F	50	4.0	0.04± 0.20
BP+6 years	M	24	20.8	0.28± 0.61
	F	25	32.0	0.40± 0.65
	M+F	49	26.5 ¹	0.41± 0.81

BP : Benzo(a)pyrene.

*Per mouse, subcutaneous injection.

** Age of fresh ginseng.

***ml in drinking water for 6 weeks.

Significantly different from BP alone group at ¹p<0.05.

year-red ginseng extracts respectively. In 4, 5 and 6 year-ginsengs, the anticarcinogenic effect was prominent (p<0.05) (Table 4).

Discussion

There are many controversial reports about the pharmacological activity of the root of *Panax ginseng* C.A. Meyer which has been used for several thousands years as a tonic in traditional oriental

Table 4. Effect of red ginseng extract on the incidence of lung adenoma in mice treated with benzo(a)pyrene

Group and treatment	Mice		Incidence (%)	Multiplicity (mean± S.D)
	Sex	Number		
Untreated control	M	26	0	0
	F	24	0	0
	M+F	49	0	0
Benzo(a)pyrene (BP, 0.5 mg)*	M	40	40.0	0.45± 0.60
	F	40	55.0	1.15± 1.70
	M+F	80	47.5	0.80± 1.32
1.5 years** (5 mg/ml)***	M	24	0	0
	F	25	0	0
	M+F	49	0	0
BP+1.5 years	M	29	34.5	0.48± 0.87
	F	30	46.7	0.50± 0.57
	M+F	59	40.7	0.49± 0.73
3 years	M	25	0	0
	F	25	4.0	0.04± 0.28
	M+F	50	2.0	0.02± 0.14
BP+3 years	M	30	23.3	0.30± 0.60
	F	30	46.7	0.80± 1.21
	M+F	60	35.0	0.51± 0.97
4 years	M	25	0	0
	F	25	0	0
	M+F	50	0	0
BP+4 years	M	29	24.1	0.31± 0.60
	F	30	36.7	0.83± 1.39
	M+F	59	30.1 ¹	0.58± 1.10
5 years	M	25	4.0	0.04± 0.28
	F	25	4.0	0.04± 0.28
	M+F	50	4.0	0.04± 0.28
BP+5 years	M	30	20.0	0.30± 0.65
	F	30	40.0	0.57± 0.82
	M+F	60	30.0 ¹	0.43± 0.75
6 years	M	25	4.0	0.04± 0.28
	F	25	4.0	0.04± 0.28
	M+F	50	4.0	0.04± 0.20
BP+6 years	M	27	22.2	0.37± 0.62
	F	30	30.0	0.37± 0.79
	M+F	57	26.3 ¹	0.37± 0.70

BP : Benzo(a)pyrene.

*Per mouse, subcutaneous injection.

** Age of fresh ginseng.

***ml in drinking water for 6 weeks.

Significantly different from BP alone group at ¹p<0.05.

medicine.

The previous paper revealed that the anticarcinogenicity of ginseng powders varied depending on the types and ages in development of lung tumor

induced by BP over 9 weeks. Namely, in the dried fresh ginseng powder treated group, a significant effect was observed only in 6 year-dried fresh ginseng. In the white ginseng powder treated group, 5 and 6 year-ginsengs showed significant inhibition of lung adenoma. In the red ginseng powder treated group, an anticarcinogenic effect was prominent from 4 year-ginseng. Therefore, we concluded that the older the age of the ginseng, the more effective its anticarcinogenicity.¹⁰⁾

In the present study the incidence of lung adenoma in dried fresh ginseng extract treated groups was reduced by 24.4%, 17.8%, 18.9%, 18.9%, 25.7% and 31.0% after co-treatment with 1.5, 3, 4, 5 and 6 year-dried fresh ginseng extracts, respectively. A significant effect was observed only in 6 year-dried fresh ginseng. In the white ginseng extract treated group, the incidence of lung adenoma induced by BP decreased by 22.5%, 0%, 0% and 35.8% after co-treatment with 3, 4, 5 and 6 year-white ginseng extracts, respectively. Only six year-ginseng showed significant inhibition of lung adenoma. In the red ginseng extract treated group, the incidence of lung adenoma induced by BP diminished by 14.3%, 26.3%, 36.6%, 36.8% and 44.6% after co-treatment with 1.5, 3, 4, 5 and 6 year-red ginseng extracts, respectively. From 4 year-ginseng, the anticarcinogenic effect was prominent. From these results, various types and ages of ginseng extract showed similar patterns of anticarcinogenicity compared with those of ginseng powders. The administration dosage of ginseng extracts would be almost the same as ginseng powders because ginseng extracts were given at a dose of 1/2 of the powders.

Therefore, the active components of ginseng which exert anticarcinogenic activity might be present in

higher percentages in older and red ginseng. However, extraction of ginseng in the water bath seemed to have no influence on its anticarcinogenic effect. In future, we plan to isolate and elucidate the active components and determine their percentage in various types and ages of ginseng.

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