



Review

# Experimental and epidemiological evidence on non-organ specific cancer preventive effect of Korean ginseng and identification of active compounds

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## Abstract

*Panax ginseng* C.A. Meyer has been the most highly recognized medicinal herb in the Orient. The prolonged administration of red ginseng extract significantly inhibits the incidence of hepatoma and also proliferation of pulmonary tumors induced by aflatoxin B<sub>1</sub> and urethane. Statistically significant anticarcinogenic effects were in aged or heat treated extracts of ginseng and red ginseng made by steaming in a 9 weeks medium-term anticarcinogenicity test using benzo[a]pyrene. In case-control studies, odds ratios (OR) of the cancer of lip, oral cavity and pharynx, larynx, lung, esophagus, stomach, liver, pancreas, ovary, and colorectum were significantly reduced. As to the type of ginseng, the ORs for cancer were reduced in user of fresh ginseng extract intakers, white ginseng extract, white ginseng powder, and red ginseng. In a cohort study with 5 years follow-up conducted in a ginseng cultivation area, ginseng users had a decreased relative risk (RR) compared with non-users. The relative risks (RRs) of ginseng users were decreased in gastric cancer and lung cancer. These findings strongly suggest that *Panax ginseng* C.A. Meyer cultivated in Korea has non-organ specific cancer preventive effects against various cancers. To investigate the active components for cancer prevention, several fractions of fresh and red ginseng and four semi-synthetic ginsenoside Rh<sub>1</sub>, Rh<sub>2</sub>, Rg<sub>3</sub> and Rg<sub>5</sub>, the major saponin components in red ginseng, were prepared among the ginsenosides. By using Yun's model, Rg<sub>3</sub> and Rg<sub>5</sub> showed statistically significant reduction of lung tumor incidence and Rh<sub>2</sub> had a tendency to decrease the incidence. In conclusion, these results strongly suggested that *Panax ginseng* C.A. Meyer cultivated in Korea is a non-organ specific cancer preventive against human cancers and also indicated that the anticarcinogenicity or human cancer preventive effect of *Panax ginseng* is due to ginsenoside Rg<sub>3</sub>, Rg<sub>5</sub> and Rh<sub>2</sub>.

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## 1. Introduction

Cancer research in Korea started later than other countries. In 1949, Dr. Il Sun Yun analyzed 632 cases of histologically diagnosed cancer patients who were

admitted between 1925 and 1939 to the Severance Medical College (the forerunner of Yonsei Medical School), and reported the finding in *Cancer Research* [1]. This paper showed findings similar to those in Western countries with no remarkable exceptions; namely, carcinomas of penile skin and liver, which were prevalent among Koreans. Subsequently, he studied the influence of splenic extract on mouse skin

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Fig. 1. *Panax ginseng* C.A. Meyer in Korea are classified into fresh ginseng (left), white ginseng (center) and red ginseng (right).

cancer induced by methylcholanthrene, and presented a series of papers. Seventeen years later, in 1966, the Korean cancer Research Association was established. In 1977, growing interests of molecular biologists, immunologists, biochemists, and medical doctors in mutagenesis and carcinogenesis research led to the birth of the Korean Society of Environmental Mutagens and Carcinogens.

In spite of incessant new discoveries and clinical application of cancer chemotherapeutics [2–4], 5 years survival rates of cancer patients were less than one in five in the 1930s, and increased to one in four in the 1940s and one in three in the 1960s. By the late 1970s, the goal of 5 years cancer survival rate was to achieve one out of two patients, but it was not successful [5]. At present, it is still only two out of five patients, corresponding to 40% of “observed survival rate”.

At that time, a few Korean scientists who realized such limitations in cancer treatment came to the conclusion that primary prevention was a means of non-toxic natural products. Since then, we have been

trying to find non-toxic cancer chemopreventives or immunopreventives in natural products which have been used historically.

Ginseng has been used as one of the most valuable natural tonics in the Orient for over 2000 years (Fig. 1) [6]. In Asia, it has been widely believed that ginseng is a miraculous medicine or mysterious tonic, improves one’s physical condition, and prolongs life with long-term administration. Considering Shenong’s report in Liang Dynasty, China, sixth century that the long-term use of ginseng led to prolongation of life span [7]. We hypothesized that the life-prolongation effect of ginseng described by Shenong might be due to potential prevention of uncontrollable diseases such as cancer that could not effectively be treated even by modern medicine. Therefore, long- and medium-term experiments, two case-control studies, cohort study based on population and identification of active compounds in ginseng were carried out to evaluate the effect of ginseng on inhibition or prevention of carcinogenesis or cancer and results obtained are summarized below.

Table 1  
Effect of red ginseng extract on pulmonary adenoma induced by various chemical carcinogens in long-term in vivo experiments

	Sacrifice (weeks)	Weight of lung	Incidence of lung adenoma	Diffuse infiltration	Incidence of hepatoma
DMBA	48	21% decrease	–	63% decrease	–
Urethane	28	–	22% decrease*	–	–
Aflatoxin B <sub>1</sub>	56	–	29% decrease	–	75% decrease

DMBA: 9,10-dimethyl-1,2-benzanthracene.

\*  $P < 0.05$ .

## 2. Pre-clinical studies on anticarcinogenicity of *Panax ginseng* C.A. Meyer

### 2.1. Long-term anticarcinogenicity study

In 1978, a long-term anticarcinogenicity experiments, lasting 67 weeks involving 2000 mice was carried out to evaluate the effect of ginseng on inhibition or prevention of carcinogenesis, induced by various chemical carcinogens such as 9,10-dimethyl-1,2-benzanthracene (DMBA), urethane, *N*-2-fluorenyl-acetamide (FAA), aflatoxin B<sub>1</sub> and tobacco smoke condensates. In the group sacrificed at 28 weeks after the treatment (urethan combined with red ginseng extract), there was 22% decrease ( $P < 0.05$ ) in the incidence of lung adenoma. In the group sacrificed at 56 weeks after birth (aflatoxin B<sub>1</sub> combined with red ginseng extract), there was 75% ( $P < 0.05$ ) decrease in the incidence of liver cancer, thus demonstrating that these natural products could offer hope for human cancer prevention (Table 1) [8,9].

### 2.2. Medium-term anticarcinogenicity test (Yun's model) on ginseng

Soon thereafter, we realized that it was necessary to develop a medium-term model for further experimentation. N:GP (S) newborn mice less than 24 h old were injected once in the scapular region subcutaneously with 0.02 ml of benzo(a)pyrene (0.5 mg suspension of BP in aqueous gelatin). After weaning, red ginseng extract made of *Panax ginseng* C.A. Meyer cultivated in Korea were administered for 6 weeks through drinking water or diets. All mice were sacrificed at the 9th week after birth and the index of lung tumor incidence was scored by the method described previously [10–13].

To verify the utility of this model, ascorbic acid, carrot,  $\beta$ -carotene, soybean lecithin, spinach, *Sesamum indicum*, *Ganoderma lucidum*, caffeine, biochanin A,

red ginseng extract (6 years old), fresh ginseng (4 years old) and 13-*cis* retinoic acid, some of which are known to have anticarcinogenic activity in various animals, were also tested. Ascorbic acid, soybean lecithin, *Ganoderma lucidum*, caffeine and red ginseng extract showed inhibition of lung tumor incidence, while fresh ginseng, carrot,  $\beta$ -carotene, spinach and 13-*cis* retinoic acid did not (Table 2) [10–15]. The observation on  $\beta$ -carotene was consistent with the negative results of ATBC trial [16], CARET trial [17,18] and Physicians' Health Study [19,20], thereby suggesting that this model for lung tumor induced by 0.5 mg of BP was useful for the screening of cancer preventive agents.

Recently, loci responsible for mouse lung tumor susceptibility have been mapped to chromosomes 6, 9, 17, and 19, while those linked to lung tumor resistance have been mapped to chromosome 4, 11, 12, and 18. Known candidate susceptibility or resistance genes include the K-ras proto-oncogene on chromosome 6 and the p16 tumor suppressor gene on chromosome 4. With evidence of considerable overlap between the genetic alterations that underline human and mouse tumorigenesis, the mouse lung tumor model has been expanded to include pre-clinical screening of chemopreventive

Table 2  
Evaluation of anticarcinogenicity using Yun's 9 weeks medium-term anticarcinogenicity model

Negative	Positive
Carrot	Ascorbic acid
Fresh ginseng (4 years old)	Soybean lecithin
Spinach	<i>Ganoderma lucidum</i>
$\beta$ -Carotene	Red ginseng extract (6 years)
<i>Sesamum indicum</i>	Caffeine
13- <i>cis</i> retinoic acid	Capsaicin
French wine	Biochanin A
Refined rice wine	2-Allylthiopyrazine
Authentic honey	

agents against human lung cancers [21]. This mouse lung tumor model has been adopted by researchers, including Chemoprevention Branch of the NCI. Furthermore, this model system also confirmed negative anticarcinogenicity effect of 9-*cis* retinoic acid, 4-HPR and oltipraz that were known to be promising cancer preventive agents in NCI recommended models [22].

### 2.3. Dependency of anticarcinogenicity on the types and ages of ginseng

Using Yun's model, we further investigated whether fresh or white ginseng had similar anticarcinogenic effects and also whether these effects were dependent on the types and ages of the ginseng. Among fresh ginseng of 1.5, 3, 4, 5, and 6 years of age, significant anticarcinogenic effects were observed with powders and extracts of 6 years dried fresh ginseng, 5 and 6 years white ginsengs and 4, 5 and 6 years red ginsengs. It was concluded from the above results that the anticarcinogenicity of ginseng varied depending on the type and age (Table 3) [23–26].

## 3. Epidemiological studies

### 3.1. Case–control study on 905 pairs

The effect of ginseng consumption on the risk of cancer was evaluated by interviewing 905 pairs of cases and controls matched by age, sex, and date

of admission to the Korea Cancer Center Hospital, Seoul. Of the 905 cases, 562 (62%) had history of ginseng intake compared to 674 of the control (75%), a statistically significant difference ( $P < 0.01$ ). The odds ratio (OR) of cancer in relation to ginseng intake was 0.56 (95% confidence interval (CI), 0.45–0.69). Ginseng extract and powder were shown to be more effective than fresh sliced ginseng, juice, or tea in reducing the OR. ORs for decreasing levels of ginseng intake were 1.00, 0.58, 0.43, and 0.25 for males and 1.00, 0.81, 0.56, and 0.52 for females. A trend test showed significant decrease in the number of cancer cases among those who reported increasing frequency of ginseng intake for males ( $P < 10^{-5}$ ) as well as for females (Table 4) ( $P < 0.05$ ). The reliability of recall for ginseng use was assessed twice by interviewing one-tenth of the randomly selected subjects using the same questionnaire. The overall agreement in reported ginseng use between the two interviews was 0.85, and the Kappa value was 0.71 ( $P < 0.01$ ). These results strongly support the hypothesis that ginseng has cancer preventive effects, as suggested by previous animal experiments [27]. The Lancet stated in an editorial in 1992 that ginseng consumption reduces the risks for all cancer types. The article included an example of the “non-organ specific approach” to cancer chemoprevention [28].

### 3.2. Case–control study on 1987 pairs

In order to further explore (a) the types of ginseng products that have the most prominent cancer

Table 3

Anticarcinogenic effects of *Panax ginseng* C.A. Meyer according to type and age; using Yun's 9 weeks medium-term anticarcinogenicity model

Experimental groups	Fresh ginseng		White ginseng		Red ginseng	
	Powder	Extract	Powder	Extract	Powder	Extract
	BP	41.3	63.9	45.0	41.3	48.6
BP + 1.5 years	31.2	48.3	–	–	37.9	40.7
BP + 3	30.0	52.5	41.3	32.0	41.7	35.0
BP + 4	31.3	51.8	38.0	46.0	31.7*	30.1*
BP + 5	30.3	47.5	31.6*	44.0	28.3**	30.0*
BP + 6	27.8*	44.1*	25.3***	26.5*	25.4***	26.3*

BP: benzo(a)pyrene. Years: age of ginseng at harvest.

\*  $P < 0.05$ .

\*\*  $P < 0.02$ .

\*\*\*  $P < 0.01$ .

Table 4

Odds ratios of cancer in ginseng intake frequency and 95% confidence intervals in 905 pairs case–control study

Frequency of ginseng intake	Male			Female		
	Cases	Controls	Odds ratios (95% CI)	Cases	Controls	Odds ratio (95% CI)
No intake	117	56	1.00	226	175	1.00
1–3 times/year	132	108	0.58 (0.38–0.90)	111	106	0.81 (0.57–1.15)
4–11 times/year	104	115	0.43 (0.28–0.67)	75	103	0.56 (0.39–0.82)
Once/month or more	83	157	0.25 (0.16–0.39)	57	85	0.52 (0.35–0.78)
Total	436	436		469	469	
Linear trend test (1 d.f.)	45.59 ( $P < 0.0001$ )			3.98 ( $P < 0.05$ )		
$\chi^2$ homogeneity test (3 d.f.)	47.28 ( $P < 0.0001$ )			16.53 ( $P < 0.001$ )		

preventive effect, (b) the reproducibility of the dose–response relationship, (c) the duration of ginseng consumption to have significant preventive effect, (d) the types of cancer that can be prevented by ginseng, and (e) the effect of ginseng on cancers associated with smoking, we increased the number of subjects for a case–control study to 1987 pairs. In this study, as with the other study ginseng users had a lower risk (OR; 0.50) for cancers compared with non-users. As for the type of ginseng, fresh ginseng extract users had 0.37 ORs for cancer, 0.57 for white ginseng extract users, 0.30 for white ginseng extract users, 0.30 for white ginseng powder users, and 0.20 for red ginseng users. Those who took fresh ginseng slices, fresh ginseng juice, and white ginseng tea, however, showed no decrease of risk. Overall, the risk decreased as the

frequency and duration of ginseng intake increased, thus showing a dose–response relationship. As for the sites of cancers, the ORs were 0.47 for cancer of lip, oral cavity, and pharynx; 0.20 for esophageal cancer; 0.36 for stomach cancer; 0.42 for colorectal cancer; 0.48 for liver cancer; 0.22 for pancreatic cancer; 0.18 for laryngeal cancer; 0.55 for lung cancer; 0.15 for ovarian cancer; and 0.48 for other cancers. In cancers of female breast, uterine cervix, urinary bladder, and thyroid gland, however, there was no association with ginseng intake (Table 5). In cancers of lung, lip, oral cavity and pharynx, and liver, smokers who took ginseng showed decreased OR compared with smokers with no ginseng intake. These findings support the view that ginseng use decreases the risk for most cancers compared to non-use [29,30].

Table 5

Odds ratios for various cancers according to ginseng intake in case–control study with 1987 pairs

Site of cancer	Cases (never taken/ever taken)	Controls (never taken/ever taken)	Odd ratios	95% CI
Lip, oral cavity, and pharynx	67/92	40/119	0.47	0.29 ± 0.76
Esophagus	40/47	14/73	0.20	0.09 ± 0.38
Stomach	142/158	76/224	0.36	0.09 ± 0.52
Colon and rectum	55/63	32/86	0.42	0.24 ± 0.74
Liver	108/156	67/197	0.48	0.33 ± 0.70
Pancreas	12/11	5/18	0.22	0.05 ± 0.95
Larynx	21/19	8/32	0.18	0.06 ± 0.54
Lung	120/156	81/195	0.55	0.38 ± 0.79
Female breast	82/92	70/109	0.63	0.40 ± 1.05
Cervix uteri	156/146	312/170	0.72	0.52 ± 1.01
Ovary	17/5	8/14	0.15	0.04 ± 0.60
Urinary bladder	23/40	16/47	0.64	0.28 ± 1.47
Thyroid gland	16/24	14/26	0.96	0.38 ± 2.44
Other	53/61	35/79	0.48	0.27 ± 0.85

Adjusted for age, sex, marital status, education, smoking, and alcohol consumption.

#### 4. Prospective study for population

Since the above promising findings were obtained at the beginning of our case–control study and also there were no human studies on the preventive effects of ginseng on cancer, we performed a more reliable cohort study in ginseng cultivation area, Kangwha-eup from August 1987 to December 1992. We studied 4634 (2362 men, 2272 women) adults over 40 years old who completed a questionnaire on ginseng intake. Among 355 (7.7%) total deaths, cancers accounted for 79 (22.8%). Subjects with cancers totaled 137 (3.0%), with 58 (1.3%) alive at the end of the study period and 79 (1.7%) deaths. Of 4634 persons eligible for analysis, 70.5% (3267) were ginseng users. Ginseng intakers had a decreased risk (RR = 0.40, 95% CI: 0.28–0.56), compared with non-intakers. On the type of ginseng, the RRs was 0.31 (95% CI: 0.13–0.74) for fresh ginseng extract users and 0.34 (95% CI: 0.20–0.53) for users of multiple combinations. There was no cancer death among 24 red ginseng intakers. There was a decreased risk with increasing frequency of ginseng intake, showing a dose–response relationship. Newly diagnosed cancer cases were identified: 42 stomach, 24 lung, 14 liver and 57 at other sites (Table 6). The RR of ginseng intakers were 0.33 (95%

CI: 0.18–0.57) in gastric cancer and 0.30 (95% CI: 0.14–0.65) in lung cancer. Among ginseng preparations, fresh ginseng extract users were significantly associated with a decreased risk of gastric cancer (RR = 0.33, 95% CI: 0.12–0.88). These results strongly suggest that *Panax ginseng* C.A. Meyer has non-toxic and non-organ specific preventive effects against cancers [31,32].

#### 5. Identification of active components in ginseng

To identify its active components, various extracts of red and fresh ginseng were tested for anticarcinogenicity using Yun's 9 weeks medium model. For fractionation of red ginseng, powdered red ginseng of 6 years old *Panax ginseng* cultivated in Korea was used for water extract, panaxadiol, panaxatriol type saponin and hexane fraction [33,34]. For the fractionation of fresh ginseng, air-dried and powdered fresh ginseng was used for 70% ethanol extract, water extract, total saponin and polysaccharide [33,35]. For the preparation of ginsenoside Rg<sub>3</sub> and Rg<sub>5</sub> mixture, the ginsenoside Rb<sub>1</sub> obtained from Korean ginseng was used [33,36]. The ginseng fractions were administered to newborn mice after weaning for 6 weeks:

Table 6  
Adjusted relative risks for selected cancers by ginseng intake in cohort study

Ginseng intake	No. of subjects	Cancers (n)								
		Stomach (42)			Lung (24)			Liver (14)		
		No.	RR	95% CI	No.	RR	95% CI	No.	RR	95% CI
No intake	1283	23	1.00	–	14	1.00	–	4	1.00	–
Ginseng intake	3167	19	0.33*	0.18–0.57	10	0.30*	0.14–0.65	10	0.86	0.25–2.94
Slices and juice	236	2	0.57	0.17–1.94	1	0.67	0.15–3.43	2	1.97	0.34–2.95
Extract	296	1	0.33*	0.12–0.88	1	0.28	0.04–2.17	–	–	–
White ginseng										
Powder	147	1	0.24	0.03–1.84	–	–	–	–	–	–
Extract	68	2	1.34	0.30–5.97	–	–	–	–	–	–
Tea	442	6	0.64	0.26–1.61	4	0.80	0.26–2.44	2	1.72	0.15–4.87
Red ginseng										
Extract	–	–	–	–	–	–	–	–	–	–
Boiled chicken with young ginseng root	381	5	0.43	0.12–1.43	1	0.35	0.08–1.95	1	0.85	0.15–4.87

RR: relative risks, adjusted for age, sex, education, smoking and alcohol consumption. CI: confidence interval. Value in parentheses indicate number of cancer cases.

\*  $P < 0.05$ .

lung adenoma incidence was 46.8% with 0.5 mg of benzo(a)pyrene. However, when treated together with red ginseng extract (2 ml/ml, drinking water) the incidence was significantly reduced to 27.5% (inhibition rate of 36.8%). Panaxadiol type saponin (67.7 ug/ml), panaxatriol type saponin (56.6 ug/ml), hexane fraction (21.9 ug/ml) and water fraction (811.4 ug/ml) showed 42.3, 41.3, 40.0 and 41.3% incidence, respectively, with no significant reduction observed [33]. The next step was to compare anticarcinogenicity of 6 years fresh ginseng fractions of 70% ethanol extract (4.72 mg/ml), water extract (6.4 mg/ml), total saponin (0.44 mg/ml) and polysaccharide (1.32 mg/ml). Lung adenoma incidence was 58.3% in 0.5 mg of BP alone treated mice. Treatment with ethanol extracts and total saponin together with BP reduced lung tumor incidence significantly to 44.1% (inhibition rate 25.7%) and 43.3% (inhibition rate 24.4%), respectively, but the incidence of polysaccharide treatment was 50.0%, thus showing no significant reduction.

A third experiment was to examine which components of red ginseng were responsible for anticarcinogenicity. For the experiment, Rg<sub>3</sub> and Rg<sub>5</sub> mixtures were selected, because they are present in large amounts in red ginseng and their semi-syntheses are possible. Lung adenoma incidence was 60.0% in 0.5 mg of BP treated mice, however, treatment with Rg<sub>3</sub> + Rg<sub>5</sub> mixture along with BP significantly reduced the incidence to 45.0% (inhibition rate 25.0%). The results showed that Rg<sub>3</sub> + Rg<sub>5</sub> had anticarcinogenic effect in Yun's medium-term model [33].

## 6. Identification of active compounds in red ginseng

### 6.1. Effective components of red ginseng

For long-term experiment, we used red ginseng extract for oral administration because it was water soluble, and found that there was significant decrease in the incidence of lung adenoma and liver cancer by red ginseng administration [8,9]. However, fresh ginseng (4 years old) was ineffective as anticarcinogenic or cancer preventive agent both in experimental animal models [11] and in human case-control [27,29] and cohort study [31]. However, when treated with heat, the fresh or white ginseng

and red ginseng were highly effective in cancer prevention.

Thirty-five kinds of ginsenosides have so far been isolated from fresh, white or red ginseng, among which 22 kinds of ginsenosides are protopanaxadiol type and 12 of them are protopanaxatriol type, and only one ginsenoside Ro is oleanane type [32]. Since ginsenosides are generally labile under acidic conditions, ordinary acidic hydrolysis is always accompanied by many side reactions such as cyclization of side chains, glycosyl elimination and epimerization of carbone-20 by SN1 reaction. Therefore, the chemical transformations of secondary metabolites occur during steaming preparative process for red ginseng. The unique components of red ginseng are known as 20(S)-ginsenoside Rg<sub>3</sub>, ginsenosides Rh<sub>2</sub>, Rs<sub>1</sub>, or Rs<sub>2</sub>, Rs<sub>3</sub>, Rs<sub>4</sub> and Rg<sub>5</sub>, plus notoginsenoside-R4 in protopanaxadiol group, and 20(R)-ginsenoside Rg<sub>2</sub>, 20(R)-ginsenoside-Rh<sub>1</sub>, ginsenoside Rh<sub>4</sub> and F4 in protopanaxatriol group. Malonyl-ginsenoside-Rb<sub>1</sub>, Rb<sub>2</sub>, Rc, and Rd are found only in white ginseng [32].

However, most of the ginsenosides are present in red ginseng in minute quantities, there, it is extremely difficult to obtain enough amount for in vivo study.

### 6.2. Nine weeks medium-term anticarcinogenicity test on ginsenosides of red ginseng

Ginsenoside Rg<sub>5</sub> was isolated as previously described [36], and Rg<sub>3</sub> and Rh<sub>2</sub> were by usual procedure from Korean red ginseng [37,38]. In brief, a mixture of 20(R)- and 20(S)-ginsenoside Rg<sub>3</sub> was obtained under mild acidic hydrolysis from protopanaxadiol saponins, ginsenoside Rb<sub>1</sub>, Rb<sub>2</sub>, Rc and Rd. The product was acetylated to give peracetates, which were further converted into 20(S)-ginsenoside Rg<sub>3</sub>, 20(R)-ginsenoside Rg<sub>3</sub>, 20(S)-ginsenoside Rh<sub>2</sub> and 20(R)-ginsenoside Rh<sub>2</sub> by direct alkaline treatment, Rh<sub>1</sub> was prepared from ginsenoside Re by similar procedure [38,39]. All of the ginsenosides obtained were identified by physicochemical and spectral analysis (IR, MASS, <sup>1</sup>H-, <sup>13</sup>C-NMR). N:GP (S). Subsequently, employing Yun's 9 weeks medium-term mouse lung tumor anticarcinogenicity test model, mice were subcutaneously injected once with 0.02 ml of BP suspension (0.5 mg, in 1% aqueous gelatin). Two control groups consisted of normal animals, (no ginseng was given) and red ginseng administered (but not BP-treated). Red ginseng

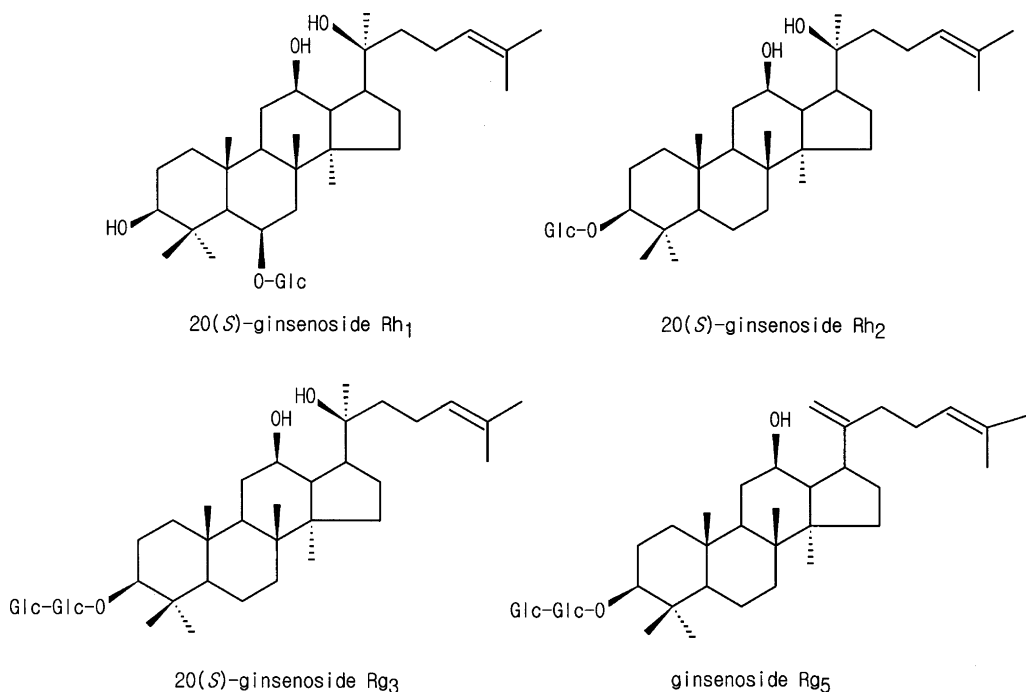


Fig. 2. Chemical structure of ginsenoside Rh<sub>1</sub>, Rh<sub>2</sub>, Rg<sub>3</sub> and Rg<sub>5</sub>. Glc-: β-D-glucopyranosyl-; Glc-Glc-: β-D-glucopyranosyl (1 → 2)-β-D-glucopyranosyl.

Table 7

Anticarcinogenicity of ginsenosides Rh<sub>1</sub>, Rh<sub>2</sub>, Rg<sub>3</sub> and Rg<sub>5</sub>, using Yun's 9 weeks medium-term anticarcinogenicity model

Experiential groups and treatment	Doses	Route	Sex	No. of mice	Incidence	Multiplicity (mean ± S.D.)
Normal control			M	25	0	0
			F	25	0	0
			M + F	50	0	0
Benzo(a)pyrene	BP: 0.5 mg/head	SC	M	25	14 (56.0)	1.20 ± 1.44
			F	25	16 (64.0)	1.80 ± 2.12
			M + F	50	30 (60.0)	1.50 ± 1.82
BP + Rh <sub>1</sub>	BP: 0.5 mg/head Rh <sub>1</sub> : 80 μg/ml	SC DW	M	30	15 (50.0)	1.20 ± 1.54
			F	30	16 (53.3)	1.49 ± 1.86
			M + F	60	31 (51.7)	1.03 ± 1.27
BP + Rh <sub>2</sub>	BP: 0.5 mg/head Rh <sub>2</sub> : 80 μg/ml	SC DW	M	30	13 (43.3)	0.77 ± 1.14
			F	30	16 (53.3)	1.53 ± 1.93
			M + F	60	29 (48.3)	1.15 ± 1.61
BP + Rg <sub>3</sub>	BP: 0.5 mg/head Rg <sub>3</sub> : 80 μg/ml	SC DW	M	30	13 (43.3)	0.67 ± 0.96
			F	30	15 (50.0)	1.03 ± 1.27
			M + F	60	28 (46.7)*	0.85 ± 1.13
BP + Rg <sub>5</sub>	BP: 0.5 mg/head Rg <sub>5</sub> : 80 μg/ml	SC DW	M	30	13 (43.3)	0.83 ± 1.21
			F	30	14 (46.7)	1.33 ± 2.89
			M + F	60	27 (45.0)*	1.08 ± 2.21

SC: subcutaneous administration; DW: drinking water.

\*  $P < 0.05$ .

extract (2 mg/ml of drinking water) was given immediately after weaning. The following ginsenosides were administered in drinking water (80 µg/ml) for 6 weeks; ginsenosides Rh<sub>1</sub>, Rh<sub>2</sub>, Rg<sub>3</sub> and Rg<sub>5</sub> (Fig. 2). Drinking water was changed every other day and diet was prepared every other week. All mice were sacrificed at the 9th week after birth, and the adenomas were counted. No lung tumor was observed in both normal control mice (no BP administered) and mice treated singularly with ginsenoside Rh<sub>1</sub>, Rh<sub>2</sub>, Rg<sub>3</sub> or Rg<sub>5</sub>. However, a lung tumor incidence of 60% was found with the group of mice which were given once with 0.5 mg of BP. On the other hand, when treated with 2 mg of red ginseng extract for 6 weeks after BP pretreatment, a 43.3% of incidence was observed (27.8% decrease), which was statistically significant. Ginsenoside Rh<sub>1</sub> (51.7% lung incidence) had no significant effect on the BP-induced lung tumor. Although ginsenoside Rh<sub>2</sub> together with BP caused 48.3% (19.5% decrease) incidence of tumor, this was considered to be a trend toward inhibition but not statistically significant.

When given with 80 µg/ml concentration for 6 weeks after BP administration, Rg<sub>3</sub> showed statistically significant decrease (22.2%) of lung tumor incidence (46.7%;  $P < 0.05$ ), whereas Rg<sub>5</sub> and BP had biologically significant 45.0% (25.0% decrease) incidence ( $P < 0.05$ ) (Table 7).

The above results obtained by Yun's model, therefore, demonstrated that, among the four ginsenosides purified from red ginseng, Rg<sub>3</sub> and Rg<sub>5</sub> yield a statistically significant reduction of lung tumor incidence, while Rh<sub>2</sub> had a tendency of decreasing the incidence. These results suggest on overall efficacy of ginseng as a cancer chemopreventive [38].

## 7. Discussion

Our strategy now to ameliorate this scourge of cancer is to switch from therapeutic approaches to chemoprevention by identifying effective natural products as chemopreventive agents. Anticarcinogenic effects of Korean red ginseng were earlier observed in 1980 by long-term [8,9] or Yun's 9 weeks medium-term experiments [11–13] with mouse lung tumor, and the anticarcinogenicity of ginseng was found to be dependent on the type and age of ginseng [23–25].

Recently, there have been many reports from various countries to support anticarcinogenicity of ginseng. Ginsenosides from *Panax notoginseng* (Sanchi ginseng) can inhibit early antigen activation of Epstein-Barr virus, and also show anticarcinogenic effects in a two-stage mouse skin model with DMBA and in lung carcinogenesis induced by 4-nitroquinolin-1-oxide [40]. Anticarcinogenic effects of majonoside from Vietnamese ginseng have also been shown in two-stage tests of mouse skin [41]. In a study of ginseng on the development of diethylnitrosamine (DEN) induced liver cancer in rats, only one of seven animals developed a tumor, when given ginseng, compared with all of the six control rats with tumor [42]. Tissue-culture biomass tincture obtained from cultured cells of *Panax ginseng* had strong inhibitory effect on rat mammary adenocarcinoma induced by methyl-*N*-nitrosourea in rats [43] and also on the development of experimental uterine cervix and vaginal tumors induced by intravaginal application of 7,12-dimethylbenz(a)anthracene (DMBA) in mice [44]. Ginseng inhibited the development of brain and spinal cord tumors induced by transplacental administration of *N*-ethyl-*N*-nitrosourea (ENU) in rats [45]. Red ginseng extracts had significant inhibitory effect on skin cancer formation in a two-stage carcinogenesis mouse model. At 50–400 mg/kg, red ginseng extract inhibited development of skin papillomas in mice induced by DMBA and croton oil, and decreased the incidence, while prolonging the latent period before tumor occurrence, and reduced tumor number per mouse in a dose-dependent manner [46].

Dietary administration of red ginseng powder in the initiation stage of carcinogenesis in the colon of rats suppressed preneoplastic lesions induced by 1,2-dimethylhydrazine; this effect was associated with suppression of cell proliferation [47]. Moreover, when red ginseng powder was delivered during the initiation phase of carcinogenesis, simultaneously with exposure to azoxymethane (AOM) only a modest inhibition of aberrant crypt foci (ACF) was noted at only 0.5 mg/kg dose of ginseng. Thus, the effect of ginseng during the initiation of ACFs from the normal mucosa is relatively weak. However, after ACFs have been established in the colon during a 4 weeks outgrowth period, an additional 4 weeks exposure to red ginseng at a dose of 2 mg/kg significantly reduced the incidence of ACFs. It was also noted that the

ginseng powder during the post-initiation time decreased the number of multiple crypt containing foci. These results suggest that some factor in red ginseng powder inhibits the growth of preneoplastic lesions in the rat colon induced by azoxymethane [48]. In MCF-7 breast-cancer cells, the ability of American ginseng to induce estrogen-regulated gene *pS2* and affects cell-cycle were assessed by northern blot analysis and by flow cytometry, respectively. Both American ginseng and oestradiol equally induced *pS2* RNA expression, but only the ginseng decreased cell proliferation ( $P < 0.005$ ) in a dose-dependent manner [49]. Oral administration of red ginseng extracts significantly suppressed spontaneous liver tumor formation in C3H/He male mice [50].

We succeeded to purify and identify four ginsenosides, including ginsenoside Rh<sub>1</sub>, Rh<sub>2</sub>, Rg<sub>3</sub> and Rg<sub>5</sub>. Among the four ginsenosides, Rg<sub>3</sub> and Rg<sub>5</sub> showed statistically significant reduction of lung tumor incidence and Rh<sub>2</sub> had a tendency of decreasing the incidence. These results strongly demonstrate that the anticarcinogenicity or human cancer preventive effect of ginseng is due to ginsenoside Rg<sub>3</sub>, Rg<sub>5</sub> and Rh<sub>2</sub> present in Korean red ginseng [38,51].

There are a few reports regarding possible mechanism of ginsenosides on cancer preventive effects. The methanol extract of heat-processed *Panax ginseng* C.A. Meyer attenuates the lipid peroxidation in rat brain homogenates and is also capable of scavenging superoxide generated by xanthine oxidase or by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in differentiated human promyelocytic leukemia (HL-60) cells. Topical application of the same extract onto back of ICR mice also suppressed TPA-induced skin tumor promotion [52]. Likewise, topical application of ginsenoside Rg<sub>3</sub>, significantly inhibited TPA-induced mouse epidermal ornithine decarboxylase activity and skin tumor promotion. Expression of cyclooxygenase-2 (COX-2) in TPA-stimulated mouse skin was markedly suppressed by Rg<sub>3</sub> pretreatment. In addition, Rg<sub>3</sub> inhibited TPA-stimulated activation of NF- $\kappa$ B and extracellular-regulated protein kinase (ERK), one of the mitogen-activated protein (MAP) kinases in mouse skin as well as cultured human breast epithelial cells (MCA-10A) [53].

In case-control studies of 905 pairs and 1987 pairs, there was noticeable decrease in cancer risk for intakers of ginseng extract compared to users of fresh

ginseng, and the decrease was greatly dependent on frequency of ginseng intake [27,29]. The OR of ginseng consumers decreased in all kinds of cancers. These results strongly support the hypothesis that ginseng has cancer preventive effects, as suggested by the earlier animal experiments. Furthermore, the results of cohort study also suggest that *Panax ginseng* C.A. Meyer has non-organ specific preventive effect against cancer [54], in support of the previous case-control studies [31].

In conclusion, ginseng has been proven to be non-organ specific cancer preventive. Since minor ginsenosides in red ginseng are shown to be active components, it is hoped that synthetic ginsenosides would be available as soon as possible and their genuine merit as cancer preventive should be tested by clinical intervention.

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