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Anticarcinogenic Effect of *Panax ginseng* C.A. Meyer and Identification of Active Compounds

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The failure to improve the five-year survival rate of cancer patients, from one in three in the 1960s to one in two in the 1970s, stimulated awareness of the importance of primary prevention of cancer. Korean investigators carried out extensive long-term anticarcinogenicity experiments with 2000 newborn mice to investigate whether *Panax ginseng* C.A. Meyer inhibited carcinogenesis induced by several chemical carcinogens in 1978. There was a 22% decrease ($p<0.05$) in the incidence of urethane induced lung adenoma by the combined use of red ginseng extract. In the group sacrificed at 56 weeks after the treatment with aflatoxin B_1, the incidence of hepatoma significantly decreased to 75% by the addition of red ginseng extract ($p<0.05$). The result showed that natural products can provide hope for human cancer prevention. By the newly established '9 week medium-term anticarcinogenicity test model of lung tumors in mice' (Yun's model), we confirmed significant anticarcinogenic effects of powders and extracts of the 6-yr-old dried fresh ginseng, 5- and 6-yr old white ginsengs, and 4-, 5-, and 6-yr old red ginseng. We also demonstrated that the anticarcinogencity of ginseng was more prominent in aged or heat treated extracts of ginseng and red ginseng made by steaming. To investigate the active components for cancer prevention, several fractions of 6-yr old fresh ginseng and red ginseng, four semi-synthetic ginsenoside Rh_1, Rh_2, Rg_3 and Rg_5, major saponin components in red ginseng, were prepared. Among the ginsenosides, Rg_3 and Rg_5 showed statistically significant reduction of lung tumor incidence and Rh_2 had a tendency of decreasing the incidence. Ginsenoside Rg_3, Rg_5 and Rh_2 were found to be active anticarcinogenic compounds. Rg_3, Rg_5 and Rh_2 are active components in red ginseng, and they prevent cancer either singularly or synergistically.

Key Words : *Panax ginseng C.A. Meyer; Long-term Anticarcinogenicity Mouse Model; Chemoprevention; Medium-term Anticarcinogenicity Mouse Model (Yun's Model); Active Ginsenoside Components*

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INTRODUCTION

Despite of great advances in early diagnosis and logical discovery of chemotherapy for cancer and of substantial advances in molecular oncology, the cure rate of most cancers remain still low. Primary cancer prevention, particularly chemoprevention, could become an increasingly useful strategy in the fight against cancer (1). Fifty years have passed since the first chemotherapeutic alkylating agent was developed, and more than a hundred clinical chemotherapeutic regimens have been developed (2, 3). Nevertheless, the total number of new cancer patients worldwide in 1985 was estimated to be 7.62 million (4). The failure to improve the 5-yr observed survival from 1 in 3 in the 1960s to 1 in 2 in the 1970s stimulated awareness of the importance of primary prevention in cancer; chemoprevention (5). Since 1977, researchers in Korea have been trying to discover non-toxic cancer chemopreventive agents from natural food products, including ginseng (6).

LONG-TERM ANTICARCINOGENICITY EXPERIMENT

It is hypothesized that the life-prolongation effect of ginseng described by Shennong (7) may be due to ginseng's efficacy in preventing development of cancers. Therefore, an investigation was carried out in 1978 to evaluate the effects of ginseng on the inhibition or prevention of carcinogenesis induced by various chemical carcinogens. Red ginseng (Fig. 1) extract (1 mg/mL of drinking water) was administered orally to the weaned mice, and chemical carcinogens, 9, 10-demethyl-1, 2-benzanthracene (DMBA, 30 μg), urethane (1 mg), N-2 fluorenylacetamide (FAA, 100 $\mu g \times 5$), aflatoxin B_1 (8 μg), or Hansando tobacco smoke condensates (320 μg) were also injected into the subscapular region of ICR mice within 24 hr after birth. Controls were comprised of three groups of ICR newborn mice: normal (100), ginseng (200), and vehicle (316). The ten experimental groups were

Table 1. Survival of ICR newborn mice injected with various chemical carcinogens at weaning in long-term anticarcinogenicity experiment

Carcinogens or vehicles	Sacrificed after birth (wk)	Dose and route	Vehicle	No. of mice injected	No. of mice at weaning	%
1% gelatin	28 and 68	0.02 mL × 1 s.c.	H_2O	199	194	97.5
DMSO	56	0.01 mL × 1 s.c.		200	192	96.0
DMBA	26 and 48	30 μg × 1 s.c.	1% gelatin	210	204	97.1
Urethane	28 and 50	1 mg × 1 s.c.	1% gelatin	200	186	93.0
FAA	25 and 68	100 μg × 5 s.c.	1% gelatin	201	178	88.6
Aflatoxin B1	56	8 μg × 1 s.c.	DMSO	200	104	52.0
Tobacco smoke condensate	67	320 μg × 1 s.c.	1% gelatin	200		

DMSO: Dimethylsulfoxide, FAA: N-2- Fluorenylacetamide, DMBA: 9, 10-Dimethyl-1, 2-benz(a)anthracene.

Table 2. Effect of red ginseng extract on pulmonary adenoma induced by various chemical carcinogens in long-term in vivo experiments

	Sacrifice (wks)	Weight of lung	Incidence of lung adenoma	Diffuse infiltration	Incidence of hepatoma
DMBA	48	21% decrease	–	63% decrease	–
Urethane	28	–	22% decrease*		
Aflatoxin B_1	56	–	29% decrease	–	75% decrease*

DMBA: 9,10-dimethyl-1,2-benzanthracene. *: $p<0.05$

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

comprised of DMBA (101), DMBA combined with ginseng (103), urethane (94), urethane combined with ginseng (92), FAA (90), FAA combined with ginseng (88), aflatoxin B_1 (50), aflatoxin B_1 combined with ginseng (47) (Table 1). In the N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) group, MNNG (3 mg) was injected subcutaneously into the backs of Wistar rats once a week for 10 weeks (6, 8). The mice and rats were autopsied immediately after sacrifice. All major organs were grossly examined and weighed, and histopathological examinations were also made. In the group sacrificed at 48 weeks after the treatment with DMBA (DMBA combined with ginseng), the incidence of diffuse infiltration of pulmonary adenoma decreased by 61% ($p<0.01$), and the average lung weight of male mice decreased by 21% ($p<0.05$). In the group sacrificed at 28 weeks after the treatment with urethane, there was 22% decrease ($p< 0.05$) in the incidence of lung adenoma by the combined treatment with ginseng. In the group sacrificed at 56 weeks after treatment with aflatoxin B_1, there was decrease in the incidence of lung adenoma (29%) and hepatoma (75%) ($p< 0.05$) by the combined treatment with ginseng (Table 2). In the groups sacrificed at 68 weeks after the treatment with FAA or 48 weeks of tobacco smoke condensate treatment, statistically no significant decrease was observed. In the group sacrificed at 27 weeks after treatment with MNNG, ginseng extract had no effect on the incidence of MNNG-induced sarcoma by the combined treatment with ginseng. These findings indicated that prolonged administration of red ginseng extract inhibited the incidence and also the proliferation of tumors induced by DMBA, urethane, or aflatoxin B_1 (6, 8), providing the hope for human cancer prevention by natural products in human.

ESTABLISHMENT OF 9 WEEK MEDIUM-TERM ANTICARCINOGENICITY TEST MODEL (YUN'S MODEL)

Soon after obtaining results of long-term experiments, we realized that it was necessary to develop a medium-term

model for further experiments to eliminate the fluctuation of experimental conditions due to long-term feeding, and to include synthetic environmental chemical carcinogens such as ben30(a)pyrene (BP). Therefore, in 1983, we embarked to establish a 9-12 weeks medium-term anticarcinogenicity test model (9). A/J, C57BL/6J, C57BR/cdJ and N:GP(S) strains of newborn mice younger than 24 hr old were injected subcutaneously with 0.5 mg or 1 mg of BP and all mice were sacrificed at the 9th week after birth. Lungs were excised and fixed in Tellyesniczky's solution (100 mL of 70% ethanol, 3 mL of formalin, 5 mL of glacial acetic acid), and the number of the adenoma were than counted by the naked eyes (Fig. 2). After counting, the lungs were embedded in paraffin, cut and then stained with hematoxylineosin. To obtain an index of tumor incidence, the percentage of tumor bearing mice per total number of mice in each group was calculated. Tumor multiplicity was defined as the average number of tumors per mouse obtained, by dividing the total number of tumors by the total number of mice per group including nontumor-bearing animals. Statistical comparison was then made using the Chi-square test for tumor incidence and Student's t-test for multiplicity. Lung adenoma incidence was 46.8% and 54.4% in N:GP(S) mice treated with 0.5 mg and 1 mg of BP, respectively. Corresponding values of A/J mice were 86.7% and 88.3%, those of C57BL/6J mice were 1.3% and 0%, and those of C57BR/ cdJ were 0%. The dose response effect of BP in A/J and N:GP(S) mice were also examined: A single injection of 40 μg of BP, which was the lowest dose in this experiment, showed 71.0% incidence of lung adenoma in A/J mice, which might be too high incidence for evaluating the anticarcinogenicity of unknown compounds. However, the dose showing a 50% tumor incidence in N:GP(S) mice was found to be 0.5 mg of BP (49.4%)

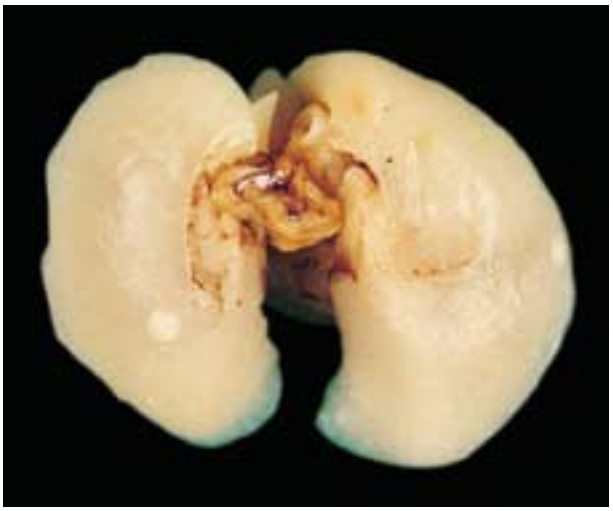

Fig. 2. Gross appearance of mouse lung with adenomas. Mouse was given a single subcutaneous injection of benzo(a)pyrene within 24 hr after birth and killed 9 weeks after birth.

(9-12). In 1983, we established a 9 week medium-term anticarcinogenicity model, termed Yun's model, based on the incidence of N:GP(S) mouse pulmonary adenomas induced by benzo(a) pyrene.

MATERIALS AND METHODS OF YUN'S MODEL

N:GP(S) newborn mice less than 24 hr old were subcutaneously injected once in the scapular region with 0.02 mL of benzo(a)pyrene (0.5 mg suspension of BP in aqueous gelatin). After weaning, test materials were administered for 6 weeks through drinking water or diets. All mice were sacrificed at the 9th week after birth. The procedures to score the index of lung tumor incidence were the same as those described under "Establishment of 9 week medium-term anticarcinogenicity test model (Yun's model)" (9-12).

EVALUATION OF YUN'S MODEL

Ascorbic acid, β-carotene, red ginseng extract (6 yr old), carrot, soybean lecithin, spinach, *Sesamum indicum, Ganoderma lucidum*, caffeine, capsaicin (13-15), fresh ginseng (4 yr old), biochanin A (16) and 2-allylthiopyrazine (17, 18) were evaluated as anticarcinogenic agents, using the above 9 week medium-term test model. Surprisingly, the authors failed to observe any anticarcinogenic effect of β-carotene, fresh ginseng, carrot, *Sesamum indicum*, spinach, however, ascorbic acid, red ginseng extract, soybean lecithin, *Ganoderma lucidum*, caffeine, capsaicin and 2-allylthiopyrazine showed positive effects (10-12, 19). This result was withheld for publication for 5 yr due to the unexplainability of the data (9). Soon after, the preliminary reports of the Physician's Health Study in U.S.A. appeared to indicate negative results with β-carotene (20). Similar results of lack of efficacy were also observed in an ATBC trial using 29,133 randomly selected male smokers (21), a CARET trial studying more than 18,000 people at high risk of lung cancer (22, 23), and a Physicians' Health Study which enrolled 22,071 American physicians (24). At the recommendation of the Chemoprevention Branch, Division of Cancer Chemoprevention and Control, US National Cancer Institute (25, 26), researchers in Korea tested the effect of red ginseng extract on azoxymethane (AOM)-induced colon cancer and N-butyl-N-(4-hydroxybutyl)-nitrosamine (OH-BBN)-induced bladder cancer, and the result were negative (unpublished). Moreover, 13-cis retinoic acid was also without benefit in the 9-week medium-term study (12, 19, 27) (Table 3).

Recently, the 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 genes for susceptibility or resistance include the K-*ras*

Table 3. Evaluation of anticarcinogenicity using Yun's 9 week medium-term anticarcinogenicity model

Anticarcinogenicity	
Negative	Positive
Carrot[28]	Ascorbic acid[27]
Fresh ginseng (4 yr old)[28]	Soybean lecithin[28]
Spinach[28]	*Ganoderma lucidum*[28]
β-Carotene[28]	Red ginseng extract (6 yr)[28]
Sesamum indicum[28]	Caffeine[28]
13-*cis* retinoic acid[30]	Capsaicin[33]
French wine[37]	Biochanin A[45]
Refined rice wine[37]	2- Allylthiopyrazine[37]
Authentic honey[37]	

Fig. 3. Fresh state of *Panax ginseng* C.A. Meyer at 1.5, 3, 4, 5, and 6 yr.

proto-oncogene on chromosome 6, and the p16 tumor supressor gene on chromosome 4. The mouse lung tumor model has been expanded by various researchers including the Chemoprevention Branch of the NCI to include preclinical screening of chemopreventive agents against human lung cancer (28). Furthermore, this model system was also employed to confirm the negative anticarcinogenicity effect of 9-cis retinoic acid, 4-HPR and oltipratz that had been known as promising cancer preventive agents in the NCI recommended model (29).

ANTICARCINOGENICITY OF TYPES AND AGES OF *PANAX GINSENG* C.A. MEYER

Using Yun's model, investigators earlier confirmed the anticarcinogenic effect of 6-yr-old red ginseng extract. In this model, we further investigated the anticarcinogenic effects of fresh or white ginsengs and their derivatives, and the dependency of their anticarcinogenic effects on types and ages of ginseng derivatives. Here, fresh ginseng of 1.5, 3, 4, 5, and 6 yr of age (Fig. 3) was dried at room temperature, finely powdered, and extracted 3 times in a water bath for 8 hr (yield of extract: 45%). White ginseng was also processed in the same way as fresh ginseng after removal of its cortex and fine root (yield of extract: 47%). For preparation of red ginseng, fresh ginseng was steamed, dried, and processed in the same way as fresh ginseng (yield of extract: 51%). Overall, dried fresh ginseng, red ginseng powders or extracts of 1.5, 3, 4, 5, and 6 yr of age, and white ginseng powders or extracts of 3, 4, 5, and 6 yr of age were used. These preparations at 5 mg/mL were orally administered at the weaning, and all the mice were sacrificed at the 9th week. The following results were obtained: 1) the incidence of BP-induced lung adenoma was 41.3%, however, its incidence was reduced in the group treated with the dried fresh ginseng powder. The incidence of lung adenoma induced by BP was reduced to 31.2%, 30.0%, 31.3%, 30.3 and 27.8% ($p<0.05$) after co-treatment with 1.5-, 3-, 4-, 5-, and 6-yr-old dried fresh ginseng powders, respectively. Thus, a statistically significant effect was observed only when treated with 6-yr-old dried fresh ginseng powder. 2) the incidence of BP-induced lung adenoma was 45.0% and its incidence decreased to 41.3%, 38.0%, 31.6% ($p<0.05$), and 25.3% ($p<0.05$) after co-treatment with 3-, 4-, 5-, and 6-yr-old white ginseng powders, respectively. Thus, anticarcinogenic effects were observed with 5- and 6-yr-old white ginseng powders. 3) the incidence of lung adenoma was 48.6% in the control group, and its incidence diminished to 37.9%, 41.7%, 31.7% ($p<0.05$), 28.3% ($p<0.05$), and 25.4% ($p<0.01$) after co-treatment with 1.5-, 3-, 4-, 5-, and 6-yr-old red ginseng powders, respectively. Therefore, anticarcinogenic effect was prominent in 4-, 5-, and 6-yr-old red ginseng powders (30). Simultaneously, each ginseng powders of various types and ages were extracted and these extracts (2.5 mg/mL) were orally administered. All the mice were sacrificed at the 9th week, and the following results were obtained: the incidence of BP induced lung adenoma was 63.9% in the control group for the dried fresh ginseng extract treated group, and its incidence was reduced to 48.3%, 52.5%, 51.8%, 47.5%, and 44.1% ($p<0.05$) after cotreatment with 1.5-, 3-, 4-, 5-, and 6-yr-old dried fresh ginseng, respectively. Statistical significance was observed only in 6-yr-old dried fresh ginseng extract. The incidence of lung adenoma induced by BP in the control group was 41.3% and were 31.0% 46.0%, 44.0%, and 26.5% ($p<0.05$) after co-treatment with 3-, 4-, 5-, and 6-yr-old white ginseng extracts, respectively, showing statistically significant effect with 6-yr-old white ginseng extract. In the control group, the incidence of lung adenoma induced by BP was 47.5% and its incidence diminished to 40.7%, 35.0%, 30.1% ($p<0.05$), 30.0% ($p<0.05$), and 26.3% ($p<0.05$) after co-treatment with 1.5-, 3-, 4-, 5-, and 6-yr-old red ginseng extract, respectively, thereby showing the statistically significant anticarcinogenic effects in 4-, 5-, and 6-yr-old red ginseng extracts. From these results, we concluded that significant anticarcinogenic effect was observed in extracts of 6-yr-old dried fresh ginseng, 6-yr-old white ginseng, and 4-, 5-, and 6-yr-old red ginseng. The results also demonstrated that the anticarcinogenicity of ginseng was more prominent in aged or heat treated extracts of fresh

Table 4. Anticarcinogenic effects of *Panax ginseng* C.A. Meyer according to type and age; using Yun's 9 week medium-term anticarcinogenicity model

Experimental groups	Incidence of lung adenoma					
	Fresh ginseng		White ginseng		Red ginseng	
	Powder	Extract	Powder	Extract	Powder	Extract
Benzo(a)yrene (BP)	41.3	63.9	45.0	41.3	48.6	47.5
BP+1.5 yr	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. *: $p<0.05$, †: $p<0.02$ and ‡: $p<0.01$.

ginseng and red ginseng prepared by steaming (30-32) (Table 4).

SUPPORTIVE ANTICARCINOGENIC EFFECTS OF *PANAX GINSENG* C.A. MEYER IN VARIOUS MODELS IN VITRO AND IN VIVO

In a study on the development of rat liver cancer induced by diethylnitrosamine, only one out of seven rats given red giseng developed a tumor, whereas all of the six control rats succumbed to tumor (33). Tissue-culture biomass tincture obtained from cultured *Panax ginseng* cells has a strong inhibitory effect on the development of rat mammary adenocarcinoma induced by methyl-N-nitrosourea administration (34) and experimental uterine cervix and vaginal tumors (35). Red ginseng extracts had a significant inhibitory effect on skin cancer formation in a two-stage carcinogenesis mouse model: red ginseng extract at 50-400 mg/kg inhibited the development of skin papillomas induced by DMBA and croton oil in mice, decreased in the incidence, prolonged the latent period before tumor occurrence, and reduced tumor number per mouse in a dose-dependent manner (36). 12-*o*-tetradecanoylphorbol-13-acetate (TPA)-induced production of tumor necrosis factor in mouse skin was inhibited by methanol extract of heat-processed neoginseng (37). 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 (38).

It should be noted here that Chinese (33, 36), Russian (34, 35), Korean (37) and Japanese (38) scientists only recently began to concentrate their effort on the cancer-preventive rather than the general effects of ginseng.

CONSTITUENTS OF *PANAX GINSENG* C.A. MEYER CULTIVATED IN KOREA

The presence of saponin in ginseng was first reported by Garriques in 1854 (39), when he isolated a saponin component from American ginseng, *Panax quinquefolius* and named it "Panaquilon". In 1957, Brekhman et al. reported saponin as the active component of ginseng (40). In 1965 the Shibata and Tanaka's group reported that ginseng saponin was triterpenoidal glycosides of dammarane type with glucose, arabinose, xylose or rhamnose, and named them ginsenoside-Rx as active components (41). Wu et al. also isolated *α*-pyrrolidone, an artifact of ginseng alkaloids extract isolated and found to suppresss the growth rate of HeLa and KB cells in 1969 (42). 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 one ginsenoside Ro is oleanane type. 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 process to prepare red ginseng. The unique components of red ginseng are known as 20(S)-ginsenoside Rg_3 (43), ginsenosides Rh_2 (43), Rs_1, or Rs_2, Rs_3, Rs_4 (44) and Rg_5 (45), plus notoginsenoside-R4 in protopanaxadiol group, and 20 (R)-ginsenoside Rg_2 (46), 20(R)-ginsenoside-Rh_1, ginsenoside Rh_4 and F_4 (47) in protopanaxatriol group. Malonyl-ginsenoside-Rb_1, -Rb_2, -Rc, and Rd are found only in white ginseng (48). Among chemical constituents other than saponin, 1-2% ether soluble components are present in the root of ginseng. Twelve kinds of phenolic compounds, including salicylic acid, caffeic acid, and maltol, have been isolated from ginseng. Especially, maltol which is present only in red ginseng and produced from maltose by amino-carbonyl reaction, shows antioxidant activity. Nine kinds of polyacetylene compounds have been isolated and characterized as pananaxynol, panaxydol, panaxytriol, acetylpanaxydol, chloropapaxydol, and panaxyne, and also ginsenoyne A, B, C, D, E, F, G, H, I, J, K from hexane-soluble fraction have been reported. As for essential oils, about 30 kinds of sesquiterpenes including azulene and patchoulene have been

identified from the ether soluble fraction of fresh ginseng, and five kinds of methoxypyrazine and eight kinds of alkypyrazine derivatives have been identified from the basic fraction of the ether-soluble extract. Sesquiterpene alcohol, panasinsanols A and B, have also been isolated. Seven kinds of β-caboline alkaloid have been isolated from the ether-soluble alkaloidal fraction, and choline has been isolated from the water soluble fraction. Twenty-one kinds of neutral or acidic polysaccharides which make up 50-60% of the ginseng root have been purified and named panaxan A-U consisting of glucose, arabinose, galactose, rhamnose, xylose or uronic acid. Ginseng contains 12 to 15% of nitrogen containing compounds, which are comprised of amino acids, adenosine, and pyroglutamic acid, and Arg-Fru-Glc is formed by amino-carbonyl reaction during the preparation of red ginseng. Other vitamins, inorganic substances, free monosaccharides, and organic acids are also present in ginseng (49).

ANTICARCINOGENICITY OF VARIOUS GINSENG FRACTIONS

Ginseng has been taken as tonic for a long time in Korea. Therefore, instead of examining its active fractions or components, we focussed our study to confirm whether ginseng has an effective anticarcinogenic agent in humans as it has been shown in rodents for more than 15 yr.

To identify its active fractions, several extracts of red and fresh ginseng were tested for anticarcinogenicity using Yun's 9 week medium model. For fractionation of red ginseng, powdered red ginseng (2 kg) of 6 yr old *Panax ginseng* C.A. Meyer cultivated in Korea was extracted with water (2 L × 2) at 90°C and filterd, and one-tenth of the combined filtrates were evaporated to give a "water extract" (104 .4 g). Remaining combined filtrates were successsively extracted with hexane (1 L × 3) and water saturated n-BuOH (700 mL × 3), and dried to give hexane fraction (1.2 g) and named panaxan A-U consisting of glucose, arabinose, galatose, rhamnose, xylose or uronic acid. The water layer was also evaporated to give water fraction (715.9 g). n-BuOH fraction was chromatographed on silica gel column, and the gel was eluted with $CHCl_3$-MeOH-H_2O (10:3:1→7:3:1). Eluates were examined by TLC together with authentic samples, and panaxadiol type saponin (29.2 g) and panaxatriol type saponin (32.8 g) were obtained (50, 51).

For fractionation of fresh ginseng, fresh ginseng was air-dried and powdered. The powdered fresh ginseng (1 kg) was extracted with water (2 L × 2) at 90°C and filtered. One-tenth of the combined filtrates were evaporated to give a water extract (49.2 g), and nine-tenths of the combined filtrates were extracted with ethyl ether (1 L × 3) and water saturated n-BuOH (700 mL × 3), to give n-BuOH fraction. The combined n-BuOH fraction were dried and evaporated under the reduced pressure to give total saponin (63.6 g).

The powdered fresh ginseng (500 g) was extracted with 70% EtOH (1 L × 3) at 80°C and filtered, and the combined filtrates were then evaporated to give EtOH extract (142.1 g). To obtain polysaccharide fraction from fresh ginseng, the air-dried and powdered fresh ginseng (1 kg) was defatted with 85% EtOH (2 L × 3), and the residues were extracted with hot water (1 L × 3). The combined extracts were evaporated to appropriate volumes and then dialyzed against running water for 3 days and distilled water for 1 day. After non-dialyzate portion was centrifuged to remove insoluble materials, the resulting supernatant was precipitated with 6 volumes of EtOH, and the precipitate was lyophilized to give polysaccharide fraction (13.3 g) (52).

For the preparation of ginsenoside Rg_3 and Rg_5 mixture, the ginsenoside Rb_1 (10 g) obtained from Korean ginseng (10 g) was hydrolyzed with 50% acetic acid (500 mL) at 70°C for 3 hr. The reaction mixture, concentrated to appropriate volume, was left at 4°C for 1 day and filtered. The filtrate was diluted with water (500 mL) and extracted with n-BuOH (250 mL × 3). The combined n-BuOH fractions were washed with saturated $NaHCO_3$ solution and evaporated under the reduced pressure. The residue was chromatographed on silica gel column, using $CHCl_3$-MeOH-H_2O (9:3:1) as solvent, to obtain ginsenoside Rg_3 and Rg_5 mixture. Ginsenoside Rg_3 and Rg_5 mixtures were subjected to HPLC (Waters 244, CLC-ODS, RI dectector), using acetonitril-water (60:40) as mobile phase, to analyze the ratio of ginsenoside Rg_3 to Rg_5 (2.6 g) (45, 50).

The ginseng fractions were administered to newborn mice after weaning for 6 weeks: Red ginseng water extract (2 mg/mL drinking water), hexane fraction (21.9 μg/mL), ether fraction (42.3 μg/mL), panaxadiol type saponin (67.7 μg/mL), panaxatriol type saponin (56.6 μg/mL) or water extract (811.4 μg/mL) in experiment 1; 70% ethanol extract of fresh ginseng (4.72 mg/mL), total saponin of fresh ginseng (0.44 mg/mL) and polysaccharide of fresh ginseng (1.32 mg/mL) in experiment 2; ginsenoside Rg_3+Rg_5 (7:3 ratio, 80 μg/mL) in experiment 3. All mice were sacrificed at the 9th week after birth.

Lung adenoma incidence was 46.8% in mice treated with 0.5mg of BP. However, when treated together with red ginseng, the incidence significantly reduced to 27.5% (inhibition rate 36.8%). Panaxadiol type saponin, panaxatriol type saponin, hexane fraction and water fraction showed 42.3%, 41.3%, 40.0% and 41.3% incidence, respectively, with no significant reduction observed in experiment 1 (Table 5).

The next step was to compare anticarcinogenicity of 6 yr fresh ginseng fractions of 70% ethanol extract, water extract, total saponin and polysaccharide. Lung adenoma incidence was 58.3% in 0.5 mg of BP alone treated mice. The treatment of ethanol extract 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, however the incidence of polysaccharide treatment

Table 5. Effects of red ginseng water extract, panaxadiol type saponin, panaxatriol type saponin, hexane fraction and water fraction on the incidence of benzo(a)pyrene induced lung tumor in mice using Yun's medium-term test model

Experiment-1					
Experimental groups and treatment	Sex	No. of mice	No. of mice with lung tumor	Lung tumor incidence (%)	Inhibition rates (%)
Benzo(a)pyrene (BP)	M	40	15	37.5	
0.5 mg/mice S.C.	F	39	22	56.4	
	M+F	79	37	46.8	Reference
BP+Red ginseng water extract	M	40	8	20.0	
2 mg/mL D.W.	F	40	14	35.0	
	M+F	80	22	27.5*	36.8
BP+Panaxadiol type saponin	M	38	16	42.1	
67.7 μg/mL D.W.	F	40	17	42.5	
	M+F	78	33	42.3	9.6
BP+Panaxatriol type saponin	M	40	16	40.0	
56.6 μg/mL D.W.	F	40	17	42.5	
	M+F	80	33	41.3	11.8
Bp+Hexane fraction	M	–	–	-	
21.9 μg/mL D.W.	F	40	16	40.0	
	M+F	40	16	40.0	14.6
BP+Water fraction	M	40	13	32.5	
811.4 μg/mL D.W.	F	40	20	50.0	
	M+F	80	33	41.3	11.8

D.W.: Drinking water, *: $p<0.05$

Table 6. Effects of ethanol extract, water extract, total saponin and polysaccharide isolated from fresh ginseng on the incidence of benzo(a)pyrene induced lung tumor in mice, using Yun's 9 week medium-term anticarcinogenicity test model

Experiment-2					
Experimental groups and treatment	Sex	No. of mice	No. of mice with lung tumor	Lung tumor incidence (%)	Inhibition rates (%)
Benzo(a)pyrene (BP)	M	30	16	53.3	
0.5 mg/mice S.C.	F	30	19	63.3	
	M+F	60	35	58.3	Reference
BP+70%EtOH extract	M	30	11	36.7	
4.72 mg/mL D.W.	F	30	15	50.0	
	M+F	60	26	43.3*	25.7
BP+Water extract	M	30	13	43.4	
6.4 mg/mL D.W.	F	29	13	44.8	
	M+F	59	26	44.1*	24.4
BP+Total saponin	M	30	13	43.3	
0.44 mg/mL D.W.	F	30	13	43.3	
	M+F	60	26	43.3*	25.7
BP+Polysaccharide	M	30	13	43.3	
1.32 mg/mL D.W.	F	30	17	56.7	
	M+F	60	30	50.0	14.2

D.W.: Drinking water, *: $p<0.05$

was 50.0%, with no significant reduction being observed in experiment 2 (Table 6).

Experiment 3 was to examine which components of red ginseng were responsible for anticarcinogenicity. For the experiment, Rg_3 and Rg_5 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 alone treated mice, however, the treatment of Rg_3+Rg_5 mixture with BP significantly reduced the incidence to 45.0% (inhibition rate 25.0%). The results showed that Rg_3+Rg_5 had anticarcinogenic effect in Yun's model (50) (Table 7).

Since red ginseng showed the most effective anticarcinogenicity, semi-synthesized ginsenoside Rg_3 and Rg_5 mixtures were selected for experiment. The results showed significant inhibition of lung adenoma in the Yun's model, indicating that ginsenoside Rg_3 and Rg_5, alone or in combination, would be active anticarcinogenic components.

Table 7. Effects of ginsenoside Rg_3+Rg_5 mixture on the incidence of benzo(a)pyrene inducecd lung tumor in mice, using Yun's 9 week medium-term anticarcinogenicity test model

Experiment-3					
Experimental groups and treatment	Sex	No. of mice	No. of mice with lung tumor	Lung tumor incidence (%)	Inhibition rates (%)
Benzo (a) pyrene (BP)	M	25	14	56.0	
0.5 mg/mice S.C.	F	25	16	64.0	
	M+F	50	30	60.0	Reference
BP+Ginsenoside Rg_3+Rg_5	M	30	13	43.3	
80 μg/mL D.W.	F	30	14	46.7	
	M+F	60	27	45.0*	25.0

S.C: Subcutaneous administration, D.W.: Drinking water, *: $p<0.05$

IDENTIFICATION OF ACTIVE ANTICARCINOGENIC COMPONENTS IN RED GINSENG

Fresh *Panax ginseng* C.A. Meyer cultivated in Korea (Korean red ginseng) was reported to be ineffective as anticarcinogenic or cancer preventive agent both in experimental animal model and in human case-control and cohort study. However, when treated with heat, the fresh or white ginseng and red ginseng were highly effective in cancer prevention. Consequently, we purified four compounds, 20(S)-ginsenoside Rh_1 (Rh_1) (54), 20(S)-ginsenoside Rh_2 (Rh_2) (55), 20(S)-gisenoside Rg_3 (Rg_3) and ginsenoside Rg_5 (55) from Korean red ginseng and tested them by Yun's model.

Ginsenoside Rg_5 was isolated as previously described (45), and Rg_3 and Rh_2 were by usual procedure from Korean red ginseng (51, 55). In brief, a mixture of 20(R)- and 20(S)-ginsenoside Rg_3 was obtained under mild acidic hydrolysis from protopanaxadiol saponins, ginsenoside Rb_1, Rb_2, Rc and Rd. The product was acetylated to give peracetates, which were further converted into 20(S)-ginsenoside Rg_3, 20(R)-ginsenoside Rg_3, 20(S)-ginsenoside Rh_2 and 20(R)-ginsenoside Rh_2 by direct alkaline treatment, while Rh_1 was prepared from ginsenoside Re by similar procedure (55). All ginsenosides obtained were identified by physicochemical and spectral analysis (IR, MASS, ^1H, ^{13}C-NMR). Thereafter N:GP(S) mice were subcutaneously injected once with 0.02 mL of BP suspension (0.5 mg, in 1% aqueous gelatin), and the following ginsenosides were administered in drinking water (80 μg/mL) for 6 weeks; ginsenosides Rh_1, Rh_2, Rg_3 and Rg_5 (Fig. 4). Two control groups consisted of normal animals (no ginseng was given) and red-ginseng administered (but no BP-treated). Red ginseng extract (2 mg/mL of drinking water) was given immediately after weaning. Drinking water was changed every other day and diet was prepared every other week. At the 9th week after birth, adenomas of the lung were counted. There was no lung tumor observed in both normal control mice (no BP administered) and mice given with ginsenoside Rh_1, Rh_2, Rg_3 or Rg_5 alone. However, 60% of lung tumor incidence was found in the group given once with 0.5 mg of BP. On the other hand, when given with

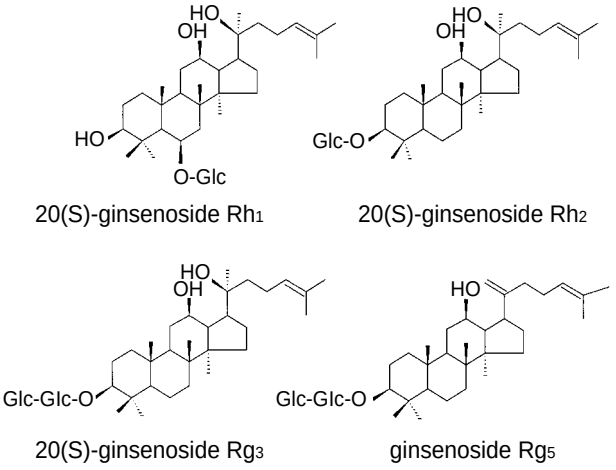

Fig. 4. Chemical structure of ginsenoside Rh_1, Rh_2, Rh_3 and Rg_5 Glc-; β-D-glucopyranosyl-, Glc-Glc-; β-D-glucopyranosyl (1→2) β-D-glucopyranosyl-.

2 mg of red ginseng extract for 6 week after benzo(a)pyrene pretreatment, 43.3% of incidence was observed (27.8% decrease), which was statistically significant. The incidence of lung adenoma showed 51.7% in mice treated with ginsenoside Rh_1, indicating no significant effect of Rh_1 on the BP-induced lung tumor. The incidence of lung tumor in mice treated with ginsenoside Rh_2 and BP showed 48.3% (19.5% decrease). Although it was not statistically significant, we considered it to represent "tendency of decrease" in the incidence.

When given with 80 μg/mL concentration for 6 weeks after BP administration, Rg_3 showed statistically significant decrease (22.2%) in lung tumor incidence (46.7%; $p<0.05$), whereas Rg_5 and BP had biologically significant incidence (45.0% and 25.0% decrease) ($p<0.05$) (Table 8).

Using Yun's model, the above results, therefore, demonstrated that, among the four ginsenosides purified from red ginseng, Rg_3 and Rg_5 revealed significant reduction of lung tumor incidence, while Rh_2 had a tendency of decreasing the incidence, indicating that ginseng is an active cancer chemopreventive agent (53).

Table 8. Anticarcinogenicity of ginsenosides Rh_1, Rh_2, Rg_3 and Rg_5, using Yun's 9 week medium-term anticarcinogenicity model

Experintal 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			M	25	14 (56.0)	1.20±1.44
	BP: 0.5 mg/head	S.C.	F	25	16 (64.0)	1.80±2.12
			M+F	50	30 (60.0)	1.50±1.82
BP+Rh_1			M	30	15 (50.0)	1.20±1.54
	BP: 0.5 mg/head	S.C.	F	30	16 (53.3)	1.49±1.86
	Rh_1: 80 μg/mL	D.W.	M+F	60	31 (51.7)	1.03±1.27
BP+Rh_2			M	30	13 (43.3)	0.77±1.14
	BP: 0.5 mg/head	S.C.	F	30	16 (53.3)	1.53±1.93
	Rh_2: 80 μg/mL	D.W.	M+F	60	29 (48.3)	1.15±1.61
BP+Rg_3			M	30	13 (43.3)	0.67±0.96
	BP: 0.5 mg/head	S.C.	F	30	15 (50.0)	1.03±1.27
	Rg_3: 80 μg/mL	D.W	M+F	60	28 (46.7)*	0.85±1.13
BP+Rg_5			M	30	13 (43.3)	0.83±1.21
	BP: 0.5 mg/head	S.C.	F	30	14 (46.7)	1.33±2.89
	Rg_5: 80 μg/mL	D.W	M+F	60	27 (45.0)*	1.08±2.21

S.C.: Subcutaneous administration, D.W.: Drinking water, *: $p<0.05$.

DISCUSSION

As early as the 1960s, alkaloid components of ginseng, α-pyrrolidone, was reported to inhibit the growth of HeLa cells (42). Thereafter, saponins have been found to have antimutagenic activity in vitro and in vivo (56); growth inhibitory activity against several tumor cell lines including nude mouse-transplantable human colon adenocarcinoma cells (MK-1 cells, mouse melanoma cells (B-16 cells), mouse fibroblast-derived tumor cells (L929 cells), human colon adenocarcinoma cells (SW620), human uterus carcinoma cells (HeLa cells) and human erythroleukemic cells (K562 cells) (57); growth inhibition of human ovarian cancer cells in nude mice (58); reverse transformation in cultured Morris hepatoma cells (59); induction of differentiation by ginsenoside in F9 teratocarcinoma cells (60); and immunomodulating activity of Rg_1 in mice (61, 62). The red ginseng was found to activate natural killer cells in mice with lung adenoma induced by urethane and benzo(a)pyrene (63). The polysaccharide revealed anticomplementary activity (64, 65), reticuloendothelial system-potentiating activity, alkaline phosphatase-inducing activity (66), and cytoprotective activity (67, 68). Lately, various polyacetylenes extracted from ginseng (69, 70) are known to have cytotoxic activity (71).

Ginsenoside Rh_1 and Rh_2 have recently been reported to cause differentiation of F9 teratocarcinoma cells, and it has been suggested that the effects of ginsenosides might have been exerted via binding with a glucocorticoid receptor or its analogous nuclear receptor (60). In nude mice bearing HRA cell tumors, oral administration of Rh_2 resulted in a significant retardation of tumor growth, consequently markedly prolonging survival time (58). The systemic as well as oral multiple administrations of ginsenoside Rg_3 inhibited lung metastasis produced by Bl6-BL6 melanoma and Colon 26-M3.1 carcinoma cells in mice. This antimetastatic effect was thought to be associated with the inhibition of the invasion and adhesion by tumor cells as well as suppression of tumor-induced angiogenesis (72-77). These results dealt mostly with induction of tumor cell differentiation, inhibition of tumor growths, prolongation of animal survival times or inhibition of metastasis. Generally, characteristics of the anticancer effect of ginseng may be summarized as follows: 1) it is observed only in slow-growing tumors such as Ehrlich and sarcoma 180 ascites tumours in vivo, 2) it is not observed in rapidly growing tumors such as L1210, P388 ascites tumors and Walker carcinosarcoma, and 3) there is no dose-response relationship and no cumulative effect (78-82).

Our strategy now is to switch from therapeutic approaches to chemoprevention of cancer by identifying effective natural products. Anticarcinogenic effects of Korean red ginseng was earlier observed in l980 by long-term (6, 8) or medium term model (Yun's model) (9-12) with mouse lung tumor. We observed that anticarcinogenicity of ginseng was dependent on the type and age of the ginseng (30, 31). In two attempts with human case-control studies (83, 84) and a cohort study (85) to evaluate the cancer preventive effect, however, fresh ginseng was found to be ineffective to decrease the relative risk (RR). On the other hand, when treated with heat, fresh ginseng, white ginseng and red ginseng were significantly effective in the decrease of RR, similar to the results obtained from animal experiments. This result suggested the generation of active cancer chemopreventive compounds of Korean ginseng by heat-treatment.

At present, 35 ginsenosides have been identified in gin-

seng, and 12 ginsenosides were found in red ginseng (86). We prepared four ginsenosides from Korean red ginseng and tested their cancer chemopreventive effect using Yun's model. This model has been successfully employed to confirm anticarcinogenicity effect of ginseng on lung tumor incidence induced by benzo(a)pyrene in mice. Mouse lung tumor model has been highly recommended for preclinical as well as clinical test models (28, 29), because this model showed no anticarcinogenicity with not only β-carotene and 13-*cis* retinoic acid (10-12, 19), but also genetic alteration in mouse lung tumor which was similarly to that of human lung cancer cells.

When taken, 4 yr-old fresh or white ginseng did not show any anticarcinogenicity in animal model (30, 31), and an epidemiological study also revealed no statistically significant reduction of the relative risk in human (83-85). When heated, however, these ginsengs were highly effective as anticarcinogenic agents and these results were confirmed by others (33-38). Red ginseng extract in a two-stage carcinogenesis mouse model had a significant inhibitory effect on skin cancer formation. At 50-400 mg/kg, red ginseng extract inhibited DMBA/croton oil-induced skin papillomas in mice, decreased the incidence, prolonged the latent period before tumor occurrence, and reduced tumor number per mouse in a dose-dependent manner (36). Recently, it has been shown that dietary administration of red ginseng powder in the initiation stage of carcinogenesis was found to suppress 1, 2-dimethylhydrazine (DMH) induced preneoplastic lesions in the colon of rats, and that this was associated with suppression of cell proliferation (38).

This fact led to search biologically active components in ginseng, and they so far identified 35 ginsenosides in general and 12 in red ginseng (86).

Some of the ginsenosides are present in red ginseng in such a minute quantity, so that it is extremely difficult to obtain the amount enough for in vivo assay. Nevertheless, we succeeded to purify and identify four ginsenosides, including ginsenoside Rh_1, Rh_2, Rg_3 and Rg_5. Among the four ginsenosides, Rg_3 and Rg_5 showed significant reduction of lung tumor incidence and Rh_2 had a tendency of decreasing the incidence. These results strongly indicate that the anticarcinogenicity or human cancer preventive effect of ginseng is due to ginsenoside Rg_3, Rg_5 and Rh_2.

There has been no report yet on the preventive effect of ginsenoside Rg_3, Rg_5 and Rh_2 on chemically induced cancer or spontaneous murine cancer in vivo. Ginsenoside Rg_5 was isolated from methanol extract of Korean red ginseng in 1996 (45), however, there is no report yet on biological activity of the compound.

Although the mechanism of how these three minor ginsenosides exhibit the anticarcinogenic effect is not known, it is highly likely that Rg_3, Rg_5 and Rh_2 in red ginseng prevent cancer either singularly or synergistically, and it is quite tempting to suggest that the ginsenosides may target one of the 5 steps of either Vogelstein's multi-stage carcinogenesis or inactivation of suppressor genes (87).

In conclusion, epidemiological studies including case-control studies (83, 84) and population based cohort study (85) proved heat-treated red ginseng to be effective non-organ specific cancer preventive (19, 85, 86, 88, 89). In order to further confirm these ginsenosides as non-organ specific cancer preventive, it is of absolute necessity to chemically synthesize large amounts of the materials for clinical testing as well.

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