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Anticarcinogenicity of Various Ginseng Fractions and Components in Red Ginseng Using Yun's Anticarcinogenicity Test Model

Taik-Koo Yun, Yun-Sil Lee, Kwang Joo Choi[1], You Hui Lee[1] and Hyo Yung Yun[2]

Laboratory of Experimental Pathology, Korean Cancer Center Hospital, Seoul, Korea
[1]Korea Ginseng & Tobacco Research Institute, Taejon, Korea, [2]Department of Surgery, College of Medicine, Chungbuk National University Hospital, Cheongju, Korea

Cancer could be conquered by early diagnosis and primary prevention through many non-toxic cancer preventives "neutraceutical" have been discovered Our study has been focused on the anticarcinogenicity of *Panax ginseng* C. A. Meyer, also known as mystic tonic, against various chemical carcinogens. Experimental and epidemiological findings showed that red ginseng and heated fresh ginseng extract had anticarcinogenic and cancer preventive effects. To identify its active components, several extracts and components of red ginseng and fresh ginseng were tested for anticarcinogenicity using Yun's 9 week medium term assay. Ethanol extract, water extract and total saponin fraction isolated from 6 year-old fresh ginseng significantly inhibited mouse lung adenoma incidence, suggesting that saponin might be the active components. Furthermore, since red ginseng among the ginseng types, showed the most effective anticarcinogenicity, semi-synthesized ginsenoside Rg3 and Rg5 mixture, major saponin components in red ginseng, were selected for further study. The results showed significant inhibition of lung adenoma in Yun's anticarcinogenicity test system, indicating that ginsenoside Rg3 or Rg5 alone or together would be active anticarcinogenic compounds.

Key Words: Anticarcinogenicity, Ginseng, Red ginseng, Ginseng fractions, Minor ginsenosides

INTRODUCTION

Since the end of 1950s,[1,2] there have been exhaustive clinical as well as experimental studies on cancer chemotherapy, immunotherapy, and combined therapy with unsatisfying results.[3] However, since 1978, it has been suggested that cancer should be conquered by prevention and it is not desirable to use synthetic agents for chemoprevention because of their toxicity problems. Therefore, attempts were made to discover non-toxic cancer preventives in natural products, and the necessity of developing new chemo- or immunopreventives from natural products, which we have been taking since time immemorial was also appreciated.

Corresponding author : Taik-Koo Yun, Laboratory of Experimental Pathology, Korean Cancer Center Hospital, 215-4, Gongneung-dong, Nowon-ku, Seoul, Korea. Tel: 02-335-1020, Fax: 02-335-1020, E-mail: tkyun@nuri.net

We hypothesized earlier that the life-prolongation effect of ginseng described by Shennong in Liang Dynasty China[4] may be due to preventive activity of ginseng against development of cancers, therefore, our study has been focused on the problem of whether ginseng has anticarcinogenicity against various chemical carcinogens, such as urethane, 9, 10-dimethyl-1,2-benzanthracene (DMBA), N-2-fluorenylacetamide (FAA), N-methyl-N'-nitroso-N-nitroguanine (MNNG), aflatoxin B_1 and tobacco smoke condensates for long-term period,[5,6] and a new 9 week medium-term anticarcinogenicity model (termed Yun's anticarcinogenicity test) using one of the environmental carcinogens, bezo(a)pyrene, was established in our laboratory to confirm and compare the anticarcinogenicity of red ginseng and compare various types and ages of ginseng.[7-10] The results showed statistically significant anticarcinogenic effects in powders and extracts of 6 years-dried fresh ginseng, 5 (powders only) and 6 years-white ginseng, and 4, 5 and 6 years-red ginseng.[11-13]

In 1987, we began an epidemiological study to confirm whether red ginseng extracts had as much anticarcinogenic effect on human beings as on mice. For this work we performed three studies; two case-control studies on cancer patients[14-17] and a cohort study on a population of a ginseng cultivation area.[18-21]

In the present study, further studies on the search of active components were performed using Yun's medium-term anticarcinogenicity test model.

MATERIALS AND METHODS

1) Plant material

The plant materials are as follows. Korean Red ginseng; *Panax ginseng* C. A. Meyer cultivated for six years, at Suwon experimental station, Korea and processed according to the GMP of Korea Tobacco & Ginseng Corporation, and Korean fresh ginseng; *Panax ginseng* cultivated under the same condition as described elsewhere[22-24] and dried without peeling.

(1) Fractionation of red ginseng

The Powered red ginseng (2 Kg) was extracted with water (2 liters $\times 2$) 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 successively extracted with hexane (1 liter $\times 3$) and water saturated n-BuOH (700 ml $\times 3$), and dried to give, hexane fraction (1.2 g) and n-BuOH fraction (68.2 g), respectively. The water layer was evaporated to give water fraction (715.9 g) also. n-BuOH fraction was chromatographed on silica gel column, using $CHCl_3$-MeOH-H_2O (10 : 3 : 1→7 : 3 : 1, gradient) as eluents. Eluates with authentic samples were examined by TLC, and panaxadiol type saponin (29.2 g) and panaxatriol type saponin (32.8 g) were fractionated.[22]

(2) Fractionation of fresh ginseng

Fresh ginseng was air-dried and powdered. The powdered fresh ginseng (1 Kg) was extracted with water (2 liters $\times 2$) at 90°C and filterd. 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 liter $\times 3$) and water saturated n-BuOH (700 ml $\times 3$), successively, to give n-BuOH fraction. The combined n-BuOH fraction were dried and evaporated under reduced pressure to give total saponin (63.6 g). The powdered fresh ginseng (500 g) was extracted with 70 % EtOH (1 liters $\times 3$) at 80°C and filtered, and the combined filtrates were then evaporated to give a 70% EtOH (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 liter $\times 3$), and the residues were extracted with hot water (1 liter $\times 3$). The combined extracts were evaporated to appropriate volumes and then dialyzed against running water for 3 days and distilled for 1 day. After non-dialyzable 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).[23]

(3) Preparation of ginsenoside Rg3 and Rg5 mixture

The ginsenoside Rb1 obtained from Korean ginseng

(10 g) was hydrolyzed with 50% aqueous acetic acid (500 ml) at 70°C for 3 hrs. The reaction mixture was concentrated to an appropriate volume and left it 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 reduced pressure. The residue was chromatographed on silica gel column, using CHCl3-MeOH-H2O (9 : 3 : 1) as solvents to obtain ginsenoside Rg3 and Rg5 mixture. Ginsenoside Rg3 and Rg5 mixture was subjected to HPLC (Waters 244, CLC-ODS, RI dectector), using acetonitril-water (60 : 40) as mobile phase to analyze the ratio of ginsenoside Rg3 and Rg5 (2.6 g).[3)]

2) Anticarcinogenicity test using Yun's 9 week medium-term model

(1) Mice: Non-inbred N : GP (S) mice were obtained from National Cancer Institute (NIH), U.S.A. Newborn mice within 24 hours after birth were used. Diet pellets were prepared according to the NIH 7-open formula diet. Male and female mice were separated and each group had 40~80 mice.

(2) Administration of carcinogen: Newborn mice less than 24 hours old were injected once subcutaneously in the scapular region with 0.02 ml of benzo (a)pyrene (0.5 mg; BP, Sigma Chemical Co., U.S.A.) suspension in aqueous gelatin. The carcinogen was used within 1 hour after emulsification.

(3) Administration of ginseng fractions: After weaning, the following ginseng fractions were admi-

Table 1. Effects of red ginseng water extract, panaxadiol type saponin, panaxatriol type saponin and hexane fraction and water extract on the incidence of lung tumor in mice induced by benzo(a)pyrene using Yun's 9 week medium-term anticarcinogenicity test model

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

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

nistered for 6 weeks: Red ginseng water extract (2 mg/ml drinking water), hexane fraction (21.9 ug/ml), ether fraction (42.3 ug/ml), panaxadiol type saponin (67.7 ug/ml), panaxatriol type saponin (56.6 ug/ml), and water extract (811.4 ug/ml) in experiment 1, fresh ginseng 70% ethanol extract (4.72 mg/ml), fresh ginseng water extract (6.4 mg/ml), fresh ginseng total saponin (0.44 mg/ml) and fresh ginseng polysaccharide (1.32 mg/ml) in experiment 2; and ginsenoside Rg3+Rg5 (7 : 3 ratio, 80 ug/ml) in experiment 3.

(4) Scoring of lung tumors: All mice were sacrificed at the 9th week after birth. Lungs were excised and fixed with Tellyesniczky's solution (100 ml of 70% ethanol, 3 ml of formalin, 5 ml of glacial acetic acid), and adenomas were then counted by the naked eye. After counting the lungs were embedded in paraffin, cut and then stained with hematoxylin-eosin. To obtain tumor incidence index, the percentage of tumor bearing mice per total number of mice in each group was calculated. Statistical comparisons were made using the Chiu-square test for tumor incidence. A null hypothesis was rejected whenever a P value of 0.05 or less was found.

RESULTS

Experiment 1: Since previous data revealed that red ginseng showed potent anticarcinogenic effect in long term and Yun's 9 week medium term systems, anticarcinogenic effects of panaxadiol type and panaxatriol type saponins, which are the major saponin of red ginseng, and hexane and water fractions of red ginseng were compared by Yun's 9 week medium term system. The mice tolerated well carcinogen and ginseng. There was no death attributable to the treatment, and overall weight gains over the 9 week period were almost same between control and treated mice (data not shown). Histopathological analysis revealed that all the lung tumors were pulmonary adenoma. Lung adenoma incidence was 46.8% in mice treated

Table 2. Effects of ethanol extract, water extract, total saponin and polysaccharide from fresh ginseng on the incidence of lung Tumor in mice induced by benzo(a)pyrene using Yun's 9 week medium-term anticarcinogenicity test model

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

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

Table 3. Effects of ginsenoside Rg3+Rg5 mixture on the incidence of lung tumor in mice induced by benzo(a)pyrene using Yun's 9 week medium-term anticarcinogenicity test model

Experiment 3					
Experimental groups and treatment		No. of mice		No. of mice with lung tumor	Lung tumor incidence (%)	Inhibition rate (%)
Benzo(a)pyrene (BP) 0.5 mg/mice S.C.	M	25	14	56.0	
	F	25	16	64.0	
	M+F	50	30	60.0	Reference
BP+Ginsenoside Rg3+Rg5 80 ug/ml D.W.	M	30	13	43.3	
	F	30	14	46.7	
	M+F	60	27	45.0*	25.0

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

with 0.5 mg of BP. When treated with red ginseng together with BP lung tumor incidence significantly reduced to 27.5% (inhibition rate 36.8%), however, with panaxadiol type saponin, panaxatriol type saponin, hexane fraction and water fraction were 42.3%, 41.3%, 40.0% and 41.3%, respectively, with no significant reduction observed.

Experiment 2: The next step was to compare anticarcinogenicity of 6 year fresh ginseng fractions such as 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 combined with BP reduced lung tumor 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% and no significant reduction was observed.

Experiment 3: Final experiment was to examine which components of red ginseng were responsible for anticarcinogenicity, and Rg3 and Rg5 mixture were selected, because they are present in red ginseng in large amount and their semi-syntheses are possible. First, lung adenoma incidence was 60.0% in 0.5 mg of BP alone treated mice. However, the treatment of Rg3+Rg5 mixture combined with BP reduced the lung tumor incidence significantly to 45.0% (inhibition rate 25.0%). On the other hand, the incidence of polysaccharide treated animal was 50.0% and no significant reduction was observed. The results showed that Rg3+Rg5 had anticarcinogenic effect in Yun's medium term assay system.

DISCUSSION

In the present study, we found that saponin components have major roles for anticarcinogenic effect in Yun's 9 week medium term assay system.

In the experiment 1, panaxatriol type saponin did not reduce lung adenoma incidence, possibly due to dosage; enough dose was not used in this experiment. In addition, since polysaccharide fraction did not inhibit lung adenoma incidence, polysaccharide fraction could be excluded from active components of anticarcinogenic effect. In the experiment 2, ethanol, water and saponin fraction exhibited anticarcinogenic effect. Because ethanol and water extracts contained large amounts of saponin, these data indicated that saponin fraction was responsible for anticarcinogenic effect of ginseng. From the above results, it is suggested that the anticarcinogenic effect of ginseng is due to saponin but not polysaccharide.

Since gene alterations of mouse lung tumor is similar to those of human,[25] the mouse lung model would be suited for preclinical and clinical model for development and testing of chemopreventive agents.[26]

Since inhibition of lung adenoma was more pronounced in red ginseng than fresh or white ginseng,[11-13] ginsenoside Rg3 and Rg5, which are rich only in red ginseng, were examined for anticarcinogenicity by using Yun's mouse lung adenoma system. The mixture of Rg3 and Rg5 showed significant inhibition of lung adenoma, suggesting that they are responsible for anticarcinogenicity of red ginseng. However, we do not know the exact nature of Rg3 and Rg5 for actual component. Nevertheless, Rg3 may have more potent anticarcinogenic capacity than Rg5, because we used the mixture of Rg3/Rg5 at the ratio of 7 to 3.

Further study is in need to elucidate mechanisms of action of these compounds. Moreover, since our epidemiological data showed that just 1~3 times intake per year significantly reduced relative risk (RR),[14,15,18] it is suggested that these components may act in oncogene activation or suppressor gene inactivation in multi-step carcinogenesis,[27] as well as in the pathway of apoptosis.

From the results of the present experimental and epidemiological studies, it is concluded that significant inhibition of lung adenoma in Yun's anticarcinogenicity test system, indicating that ginsenoside Rg3 or Rg5 alone or together would be active anticarcinogenic compounds in red ginseng.

Panax ginseng C. A. have shown anticarcinogenic and cancer preventive effect and in the present study, we also suggested that saponin may be one of active components. We think ginseng should be recognized as a functional food for cancer prevention, and that further studies for the identification of its active components and mechanisms of action, expecially focused on red ginseng.

We, therefore, further suggest that further studies on the anticarcinogenicities of individual minor ginsenosides in red ginseng and mechanism of action should be focused.

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