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Cancer Chemopreventive Compounds of Red Ginseng Produced from *Panax ginseng* C.A. Meyer

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Abstract : Fresh *Panax ginseng* C.A. cultivated in Korea (Korean red ginseng) was found to be ineffective as anticarcinogenic or cancer preventive in experimental animal model or in human case-control and cohort study. However, when treated with heat, the fresh ginseng, white ginseng and red ginseng were highly effective cancer preventives. Four compounds including 20(*S*)-ginsenoside Rh_1 (Rh_1), 20(*S*)-ginsenoside Rh_2 (Rh_2), 20(*S*)-gisenoside Rg_3 (Rg_3) and ginsenoside Rg_5 were consequently purified from Korean red ginseng, and they were tested by Yun's 9 week medium-term anticarcinogenicity test model. Rg_3 and Rg_5 statistically significantly decreased the incidence of benzo(a)pyrene-induced mouse lung tumor, Rh_2 showed tendency of decrease, and Rh1 showed no effect. It is, therefore, concluded that Rg_3 and Rg_5 are active anticarcinogenic components in red ginseng and they either singularly or synergistically act in the prevention of cancer.

Key words : Cancer chemoprevention, benzo(a)pyrene, lung tumor, *Panax ginseng*, Korean ginseng, gisenoside Rg_3, ginsenoside Rg_5

INTRODUCTION

Anticarcinogenic effects of Korean red ginseng on mouse lung tumor was earlier observed in 1980 by long-term (1, 2) and Yun's 9 week medium-term experiments (3-6). Anticarcinogenicity of ginseng was dependent on the type and age of the herb (7-9). In two attempts to evaluate the cancer preventive effect with human case-control studies (10, 11) and a cohort study (12), however, fresh ginseng was found to be ineffective in decreasing the relative risk (RR). On the other hand, when treated with heat, fresh ginseng, white ginseng and, red ginseng extracts were significantly effective in decreasing RR, similar to the results obtained with animal experiments. This result suggested the generation of active cancer chemopreventive compounds by heat-treatment.

Since then, 35 ginsenosides have been identified in ginseng, and 12 ginsenosides were found in red ginseng (13). We prepared four ginsenosides minutely present in Korean red ginseng and tested their cancer chemopreventive effect with Yun's 9 week medium-term anticarcinogenicity test model (3-6). The result obtained is described herein.

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MATERIALS AND METHODS

1. Plant material

Red ginseng of *Panax ginseng* C. A. Meyer cultivated in Korea was purchased from Korea Ginseng Corporation, Taejon, Korea.

2. Animals and carcinogen.

N; GP(S) mice were obtained from the National Cancer Institute (NCI, USA) and newborn mice within 24 hours after birth were used. Thirty each male and female mice were grouped together, and diet pellets were formulated according to the NIH 7-open formula (3-6). Chemical carcinogen used in the experiment was benzo(a)pyrene (BP) purchased from Sigma Chemical Co., USA

3. Preparation of minor ginsenosides

Ginsenoside Rg_5 from Korean red ginseng was isolated as previously described (14), and Rg_3 and Rh_2 were pre-

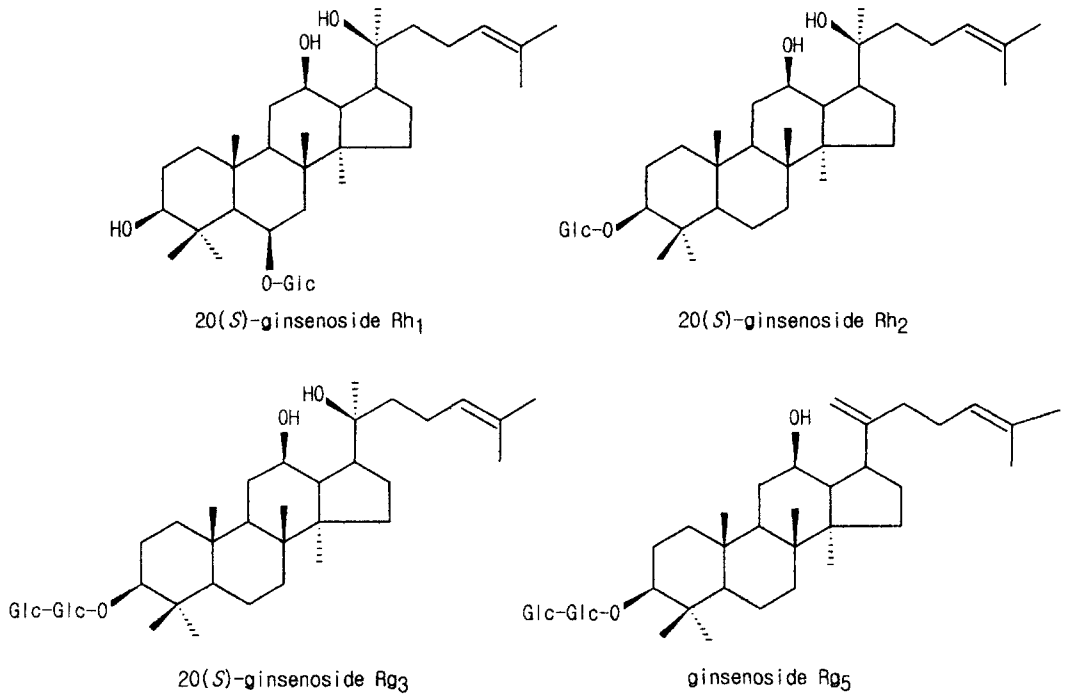

Fig. 1. Chemical structure of ginsenoside Rh_1, Rh_2, Rg_3 and Rg_5.
Glc-; β-D-glucopyranosyl-, Glc-Glc-; β-D-glucopyranosy(1→2)-β-D-glucopyranosyl-

pared by usual procedure (15). 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 alkaline treatment. Rh_1 was prepared from ginsenoside Re by similar procedure (16). All of the ginsenosides obtained were identified by comparison with authetic samples by physicochemical and spectral analysis (IR, MS, ^1H, ^{13}C-NMR) (Fig. 1).

3. Anticarcinogenicity assay by Yun's 9 week medium-term anticarcinogenicity test model (3-8)

N : GP(S) mice were subcutaneously injected once with 0.02 m*l* of BP suspension (0.5 mg, in 1% aqueous gelatin) within 24 hours after birth. Two control groups consisted of normal animals (no ginseng was given) and red-ginseng administered (but no BP-treated). Red ginseng extract (2 mg/m*l* of drinking water) and the ginsenosides Rh_1, Rh_2, Rg_3 and Rg_5 were administered in drinking water (80 μg/m*l*) immediately after weaning for 6 weeks. Drinking water was changed every other day and diet was prepared every other week.

4. Scoring of lung tumor

All mice were sacrificed at the 9th week after birth. Lungs were excised and fixed in Tellyesniczky solution (100 m*l* of 70% ethanol, 3 m*l* of formalin, 5 m*l* of glacial acetic acid). Then the adenomas were counted by the naked eye. After counting, the lungs were embedded in paraffin, cut and then stained with hematoxylin-eosin. 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 comparisons were made using the Chi-square test for tumor incidence and Students t test of multiplicity. A null hypothesis was rejected whenever a P value of 0.05 or less was found.

RESULTS

There was no lung tumor observed in both normal control mice (no BP administered) and mice given singularly with ginsenoside Rh_1, Rh_2, Rg_3 or Rg_5. However, 60% of lung tumor incidence was found with the group of mice which were given once with 0.5 mg of BP. On the other

hand, when given with 2 mg of red ginseng extract for 6 week after benzo(a)pyrene pretreatment, 43.3% of incidence was observed (27.8% decrease), which is statistically significant. Ginsenoside Rh_1 with 51.7% lung incidence indicated no significant effect on the BP-induced lung tumor. Although ginsenoside Rh_2 and BP had 48.3% (19.5% decrease) incidence of tumor, it was taken to show "tendency of decreased incidence" but was not statistically significant.

When given with 80 μg/m*l* concentration for 6 weeks after BP administration, Rg_3 showed statistically significantly decreased incidence (22.2%) of lung tumor (46.7%; $p<0.05$), whereas Rg_5 and BP had significant 45.0% (25.0% decrease) incidence ($p<0.05$).

Using Yun's 9 week medium-term anticarcinogenicity test model, the above results, therefore, demonstrated that,

Table 1. Effects of ginsenosides Rh_1, Rh_2, Rg_3, Rg_5 and red ginseng extract on the incidence of lung Tumor in mice induced by benzo(a)pyrene

Experimental groups and treatment	Dose	Sex	No. of mice	Incidence	Multiplicity (Mean ± S.D.)
Normal control	-	M	25	0	-
		F	25	0	-
		M+F	50	0	-
Red ginseng extract	2 mg/m*l*	M	25	0	-
		F	25	0	-
		M+F	50	0	-
Ginsenoside Rh_1	80 μg/m*l*	M	25	0	-
		F	25	0	-
		M+F	50	0	-
Ginsenoside Rh_2	80 μg/m*l*	M	25	0	-
		F	25	0	-
		M+F	50	0	-
Ginsenoside Rg_3	80 μg/m*l*	M	25	0	
		F	25	0	-
		M+F	50	0	-
Ginsenosiden Rg_5	80 μg/m*l*	M	25	0	-
		F	25	0	-
		M+F	50	0	-
Benzo(a)pyrene(BP)	0.5 mg/head	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+red ginseng extract	0.5 mg/head	M	30	11 (36.7)	0.57 - 0.97
	2 mg/m*l*	F	30	15 (50.0)	1.43 - 2.08
		M+F	60	26 (43.3)*	1.00 - 1.67
BP+ginsenoside Rh_1	0.5 mg/head	M	30	15 (50.0)	1.20 - 1.54
	80 μg/m*l*	F	30	16 (53.3)	1.49 - 1.86
		M+F	60	31 (51.7)	1.03 - 1.27
BP+ginsenoside Rh_2	0.5 mg/head	M	30	13 (43.3)	0.77 - 1.14
	80 μg/m*l*	F	30	16 (53.3)	1.53 - 1.93
		M+F	60	29 (48.3)	1.15 - 1.61
BP+ginsenoside Rg_3	0.5 mg/head	M	30	13 (43.3)	0.67 - 0.96
	80 μg/m*l*	F	30	15 (50.0)	1.03 - 1.27
		M+F	60	28 (46.7)*	0.85 - 1.13
BP+ginsenoside Rg_5	0.5 mg/head	M	30	13 (43.3)	0.83 - 1.21
	80 μg/m*l*	F	30	14 (46.7)	1.33 - 2.89
		M + F	60	27 (45.0)*	1.08 - 2.21

*P<0.05, M: Male, F: Female, BP: Benzo(a)pyrene Head: per mouse head

among the four ginsenosides purified from red ginseng, Rg_3 and Rg_5 revealed statistically significant reduction of lung tumor incidence, while Rh_2 had a tendency of decreasing the incidence and Rh_1 had no effect, thereby indicating overall efficacy of ginseng as a cancer chemopreventive (Table 1).

DISCUSSION

Yun's 9 week medium-term anticarcinogenicity test model (3-7), which was designed 20 years ago, has been successfully employed to confirm anticarcinogenicity effect of ginseng on benzo(a)pyrene induced mice lung tumor (1). It should be noted that mouse lung tumor model has been highly recommended for preclinical as well as clinical models (17, 18), because this model showed no anticarcinogenicity with β-carotene and 13-*cis* retinoic acid (6, 18, 19) and also genetic alteration found in mouse lung tumor was very similar to that observed in human lung cancer cells.

When taken fresh, 4 year-old fresh or white ginseng did not show any anticarcinogenicity in animal model (7-9), and an epidemiological study also revealed no statistically significant reduction of human relative risk (10-12). When heated, however, these ginsengs were highly effective as anticarcinogenic agents, and these results were confirmed by others (20, 21). 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 (20). 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 (21). These observation prompted scientific communities to frantically search biologically active components in ginseng, and led to the identification of 35 ginsenosides in general and 12 in red ginseng (13).

Some of the ginsenosides are present in red ginseng in such a minute quantity, so that it is extremely difficult to obtain enough amount 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 statistically significant reduction of lung tumor incidence and Rh_2 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_3, Rg_5 and Rh_2 present in Korean red ginseng.

There has not yet been any report on the preventive effect of ginsenoside Rg_3, Rg_5 and Rh_2 on chemically induced cancer or spontaneous murine cancer *in vivo*. Only ginsenoside Rh_1 and Rh_2 have been reported to cause differentiation of F9 teratocarcinoma cells and been suggested that the effects of ginsenosides might be due to its binding with a glucocorticoid receptor or its analogous nuclear receptor (22). 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 (23). The systemic as well as oral multiple administration of ginsenoside Rg_3 inhibited lung metastasis produced by Bl6-BL6 melanoma and Colon 26-M3.1 carcinoma cells in mice, and the antimetastatic effect was associated with inhibition of invasion and adhesion by tumor cells as well as suppression of tumor-induced angiogenesis (24). These results dealt mostly with induction of tumor cell differentiation, prolongation of animal survival times or inhibition of metastasis. Ginsenoside Rg_5 was isolated from methanol extract of Korean red ginseng in 1996 (14), however, there has been no previous reports on biological activity of the compound.

Although the mechanism of how these three minor ginsenosides exhibits the anticarcinogenic effect is not clearly understood, it has been suggested that the ginsenosides may target one of the 10 steps of either Vogelstein's multistage carcinogenesis or inactivation of suppressor genes (25).

In particular, three epidemiological studies including case-control studies (10, 11) and population based cohort study (12) proved heat-treated red ginseng to be effective non-organ specific cancer preventive. In order to further confirm these three ginsenosides as non-organ specific cancer preventive, it is of absolute necessity to chemically synthesize large amounts of the materials for clinical testing.

요 약

발암물질을 투여하여 발생하는 마우스 폐선종은 홍삼추출물의 투여에 의하여 그발생율이 억제되나 수삼을 투여하면 발생율이 억제되지 않는다. 또한 암환자-대조군연구 결과에 있어서도 수삼즙 또는 수삼절편을 복용한 사람에서는 암의 위험비가 감소되지 않

으나 수삼열탕 또는 홍삼을 복용하면 현저한 위험비의 감소를 볼 수 있었다. 이와같은 결과는 열로 처리된 홍삼중에 암예방 유효성분이 있을 것이라고 추정되어 왔다. 저자들은 4종의 홍삼중의 진세노사이드 즉 Rh_1, Rh_2, Rg_3 및 Rg_5를 고려홍삼으로 부터 분리 합성하여 윤의 9주 중기 항발암실험법에 의하여 항발암성을 관찰한 바 진세노사이드 Rg_3와 Rg_5의 투여시에는 통계학적으로 유의한 폐선종 발생율이 감소되었으나 Rh_2에서는 폐선종발생율이 약간 감소되는 경향을 보였고 Rh_1에서는 전혀 영향을 주지 않았다. 이와같은 소견으로 홍삼에 의한 항발암작용 또는 암예방작용은 홍삼중의 진세노사이드 Rg_3 및 Rg_5가 유효성분임을 파악하였으며 이들이 진세노사이드 Rg_3, Rg_5 및 Rh_2 가 단독 또는 복합적으로 작용할 것으로 추정된다.

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