

Cancer Chemoprevention by Ginseng in Mouse Liver and Other Organs

Oral administration of red ginseng extracts (1% in diet for 40 weeks) resulted in the significant suppression of spontaneous liver tumor formation in C3H/He male mice. Average number of tumors per mouse in control group was 1.06, while that in red ginseng extracts-treated group was 0.33 ($p < 0.05$). Incidence of liver tumor development was also lower in red ginseng extracts-treated group, although the difference from control group was not statistically significant. Anti-carcinogenic activity of white ginseng extracts, besides red ginseng extracts, was also investigated. In the present study, the administration of white ginseng extracts was proven to suppress tumor promoter-induced phenomena *in vitro* and *in vivo*. It is of interest that oral administration of the extracts of Ren-Shen-Yang-Rong-Tang, a white ginseng-containing Chinese medicinal prescription, resulted in the suppression of skin tumor promotion by 12-*o*-tetradecanoylphorbol-13-acetate in 7,12-dimethylbenz[*a*]anthracene-initiated CD-1 mice. These results suggest the usefulness of ginseng in the field of cancer prevention.

Key Words : Ginseng; Cancer Chemoprevention; Suppression of Tumor Promotion; Liver Neoplasms; Skin Neoplasms

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INTRODUCTION

Ginseng has been used as a medicinal plant for more than 2000 yr in Asian countries. In fact, various pharmacological activities have been found in ginseng extract or its constituents. Among them, anti-carcinogenic effect seems to be especially important, since development of effective method for cancer prevention is urgent problem not only in Asian countries but also in all over the world. Inhibitory effect of red ginseng extracts on pulmonary carcinogenesis has already been well documented (1). And tendency of suppression on liver carcinogenesis has also reported (1). Thus, at first, we have extended the study on inhibitory effect of red ginseng extracts in liver carcinogenesis using other experimental models. Furthermore, anti-carcinogenic activity of white ginseng extracts, besides red ginseng extracts, was also investigated.

MATERIALS AND METHODS

Materials

Extracts of ginseng (*Panax ginseng* C.A. Meyer) were prepared as dried powder from the dilute ethanol extracts, and provided by Wakunaga Pharmaceutical Co., Ltd. (Hiroshima, Japan). In some cases, dried sample prepared from hot water extract of ginseng was used. Dried preparation of Ren-Shen-Yang-Rong-Tang extract was also prepared from its hot water extract. Ren-Shen-Yang-Rong-Tang, a Chinese medicinal prescription, is consisted of ginseng (Renshen) and other 11 Chinese drugs (Baizhu, Ganjiang, Guizhi, Huangqi, Danggui, Chenpi, Fuling, Shudihuang, Shaoyao, Wuweizi and Yuanzhi).

7,12-Dimethylbenz[*a*]anthracene (DMBA) was purchased from Wako Pure Chemical Industries (Osaka, Japan). 12-*o*-

tetradecanoylphorbol-13-acetate (TPA) was obtained from Sigma-Aldrich Corporation (St. Louis, MO, U.S.A.).

In vivo mouse spontaneous liver carcinogenesis experiment

C3H/He male mice, which have a high incidence of spontaneous liver tumor development, were used. Mice were purchased from Charles River Japan (Tokyo, Japan). Red ginseng extracts were administered orally. Mice were killed at experimental week 40 by cervical dislocation, and the number of liver tumor nodules was counted.

In vivo two-stage mouse skin carcinogenesis experiment

CD-1 female mice (purchased from Charles River Japan, Tokyo, Japan) had their backs shaved with an electric mouse hair clipper. Initiation was accomplished by a single application of DMBA (585 nmol) on the shaved area. TPA, at the dose of 3.24 nmol, was applied twice a week starting 1 week after initiation. The experiment was continued for 18 weeks.

Effect on TPA-induced Epstein-Barr virus activation in Raji cells

Effect on Epstein-Barr virus (EBV)-early antigen (EA) induction by TPA was examined using Raji cells, which is EBV genome-carrying human lymphoblastoid cell line. Cells were incubated for 48 hr in 1 mL of culture medium containing n-butyric acid (4 mM), TPA (32 nM), and test compound or its vehicle. Smears were made from cell suspension. The cells were stained with EBV-EA positive sera obtained from nasopharyngeal carcinoma patients, and detected by a conventional indirect immunofluorescence technique. In each assay, at least 500 cells were counted and the experiments were repeated twice. The average EA induction was compared with that of the positive control experiment with n-butyric acid (4 mM) plus TPA (32 nM).

RESULTS

Inhibitory effect of red ginseng extracts on liver carcinogenesis

Effect of oral administration of red ginseng extracts on liver carcinogenesis was studied using the experimental model of spontaneous liver carcinogenesis in C3H/He male mice.

Dried powder of red ginseng extracts prepared from dilute ethanol extract was mixed into diet at the final concentration of 1%.

Consumption of diet in each group was almost the same,

Table 1. Effect of red ginseng extracts on spontaneous liver carcinogenesis in C3H/He male mice

Group	(n)	Tumor-bearing mice (%)	Number of tumors per mouse (mean ± SE)
Control	(16)	43.8	1.06 ± 0.37*
+Red ginseng extracts	(12)	33.3	0.33 ± 0.14*

* $p < 0.05$ (Student's t-test).

Table 2. Effect of white ginseng extracts on TPA-induced expression of Epstein-Barr virus early antigen in Raji cells

Concentration of white ginseng extracts (mg/mL)	Inhibition %
0 (Control)	0
1	0
10	31.7
50	65.1
100	100

and no significant difference in final body weight was observed (33.6 ± 0.5 g in control group, and 34.4 ± 0.6 g in red ginseng extracts administered group, respectively).

As shown in Table 1, the mean number of liver tumors was significantly decreased by administration of red ginseng extracts as compared with that in the control group; the control group developed 1.06 tumors/mouse, whereas the 1% red ginseng extracts treated group had 0.33 tumors/mouse ($p < 0.05$, Student's t-test). The incidence of tumor development was also lower in red ginseng extracts treated group than in control group, although the difference from control was not statistically significant. There were no differences in histological findings of tumors among the groups. The tumor cells were arranged in thin trabecular structures surrounded by sinusoidal structures. The nuclei of the tumor cells were slightly enlarged, and mitoses were seen sporadically, but pleomorphism was not so profound and the tumor was identified as well-differentiated hepatocellular carcinoma.

Anti-tumor promoting effect of white ginseng extracts

In order to evaluate the anti-carcinogenic activity of white ginseng, besides red ginseng, we have examined the potency of white ginseng extracts to suppress tumor promoter-induced phenomena in vitro and in vivo.

As shown in Table 2, white ginseng extracts suppressed EBV-EA expression in Raji cells induced by TPA, a typical tumor promoter. Inhibitory effect of white ginseng extracts was observed in a dose-dependent manner, and complete suppression was achieved at the concentration of 100 μ g/mL.

These results of in vitro study prompted us to examine in vivo anti-tumor promoting activity of white ginseng extracts. As shown in Table 3, skin tumor promotion by TPA was suppressed by oral administration of white ginseng extracts

Table 3. Effect of white ginseng extracts and Ren-Shen-Yang-Rong-Tang extracts on skin tumor promotion by TPA in DMBA-initiated CD-1 female mice

Group	(n)	Tumor-bearing mice (%)	Number of tumors per mouse (mean \pm SE)
Control	(15)	100	15.00 \pm 2.38*
+White ginseng extracts (0.04% in drinking water)	(15)	86.6	10.53 \pm 2.34
+Ren-Shen-Yang-Rong-Tang extracts (0.1% in drinking water)	(14)	64.3	3.00 \pm 0.93*

* $p < 0.01$ (Student's t-test).

(in this case, dried sample prepared from hot water extracts was used), although the effect was not statistically significant. It is of interest that the administration of the extracts of Ren-Shen-Yang-Rong-Tang, one of the white ginseng-containing Chinese medicinal prescriptions, resulted in significant suppression of skin tumor promotion.

DISCUSSIONS

Oral administration of red ginseng extracts (1% in diet) was proven to suppress mouse liver carcinogenesis. As mentioned above, tendency of anti-liver carcinogenic activity of red ginseng extracts has already been reported (1). Thus, usefulness of red ginseng in liver cancer prevention is well confirmed.

Since white ginseng, besides red ginseng, is also widely used, we have examined the anti-carcinogenic activity of white ginseng extracts. In the present study, anti-tumor promoting activity was investigated. As the results, the administration of white ginseng extracts was proven to suppress TPA-induced phenomena in vitro and in vivo.

Anti-tumor promoting activity of white ginseng extracts is now evaluating using experimental model of two-stage carcinogenesis in lung, besides skin. In a preliminary experiment, white ginseng extracts showed inhibitory effect on lung tumor promotion in a dose-dependent manner. In 4-nitroquinoline 1-oxide-initiated and glycerol-promoted lung carcinogenesis experiment, the effect of white ginseng extracts (oral administration during tumor promoting stage, added into drinking water), was apparent at the dose of 0.04%. And statistically significant suppression was observed at the dose of 0.1%.

Administration of red ginseng extracts at the dose of 0.1% in drinking water was also effective to suppress lung tumor promotion. As mentioned above, significant anti-lung carcinogenic activity of red ginseng extracts has already been reported (1). Thus, usefulness of red ginseng in lung cancer prevention is well confirmed.

From these results, both of red and white ginseng seem to be valuable in cancer control.

Since various species of ginseng, such as *Panax japonicus* C.A. Meyer (Japanese ginseng, Bamboo ginseng), *Panax notoginseng* (Burk) F. H. Chen (Sanchi-ginseng), *Panax vietnamensis* Ha et Grusho (Vietnamese ginseng), besides *Panax ginseng* C.A. Meyer, are used as medicinal plant in the world, it is of interest to evaluate these ginseng products.

Identification of active principles in red and white ginseng is also important. Various kinds of ginsenosides were already isolated, and some of them have been evaluated. For example, ginsenosides Rb1 and Rb2 were proven to have anti-tumor promoter activity in vitro. Furthermore, oral administration of ginsenoside Rb1-rich fraction resulted in strong suppression of mouse skin tumor promotion (unpublished data).

Recently, we have also identified majonoside-R2 as an anti-tumor promoting principle in Vietnamese ginseng (2). Among seven saponins isolated from this plant, majonoside-R2 showed the strongest inhibitory effect on EBV-EA expression induced by TPA in Raji cells. And majonoside-R2 was further proven to show potent anti-tumor promoting activity in two-stage skin carcinogenesis.

Ginseng-containing Chinese medicinal prescriptions are also worthy to investigate their anti-carcinogenic potency more intensively. In this paper, anti-tumor promoting activity of Ren-Shen-Yang-Rong-Tang is demonstrated in two-stage carcinogenesis of mouse skin. Combination of active principles in ginseng and other drugs in this prescription may synergistically strengthen the anti-carcinogenic activity. In fact, gomisin A isolated from Wuweizi, a component of this prescription, has already found to suppress two-stage mouse skin carcinogenesis (3).

In conclusion, usefulness of ginseng in the field of cancer prevention has been suggested experimentally, and further investigation including clinical trials should be continued.

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