Chemopreventive Effects of Ginseng on Rat Carcinogenesis

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Abstract

The chemopreventive effects of ginseng on rat carcinogenesis models were investigated. In the present study, the inhibitory effects of white and red ginseng on tumor development were examined using medium-term liver, initiation and medium-term multi-organ carcinogenicity bioassay systems. No modifying potential of the ginsengs was evident in terms of the numbers or areas of glutathione S-transferase placental form (GST-P)-positive foci, which is a marker of preneoplastic lesion in rat livers. However, white ginseng, but not red ginseng was found to decrease the incidences of adenocarcinoma of the small intestine and colon in the medium-term multi-organ carcinogenesis model. These results indicate that white ginseng may have inhibitory effects on progression stage of rat intestinal carcinogenesis, but the influence is not strong. Ginseng is unlikely to have promoting or inhibitory effects in other organs under the present type of experimental conditions. Possible application on ginseng for chemoprevention of colon cancer in humans, can be concluded given the lack of obvious adverse effects.

Keywords: Ginseng; Chemoprevention; Carcinogenesis; Medium-term assay; Colon

Introduction

Ginseng (roots of *Panax ginseng*) is well known as a traditional medicine in Asian countries. It is classified into three types depending on how it is processed; fresh ginseng: white ginseng (dried after peeling); and red ginseng (steamed and dried). Both white and red ginseng are commonly used, each type being ingested in various forms, for example as a juice, extract,

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powder, tea, tablet or capsule. Ginseng is taken not only as a medicine but aiso in dietary therapy as a health-giving vegetable with several pharmacological activities reported (1, 2, 3).

Chemopreventive effects of ginseng in human cancer have been documented (4,5). Yun and Choi have reported a case-control study in which ginseng intake was associated with a decreased risk for most cancers including carcinomas of the esophagus, stomach, colon, pancreas, lung and liver (5). Experimentally, several investigations have shown inhibitory effects of ginseng on carcinogenesis, tumor growth and metastasis (6,7,8,9,10). For example a medium-term (9 weeks) model system revealed anti-carcinogenic activity of ginseng extract against pulmonary adenoma induction by benzo(a)pyrene in newborn mice (11). Xiu-gan et al. (12) described the inhibitory effects on the development of liver cancers in diethylnitrosamine-treated rats. Recently, we have shown that dietary administration of red ginseng concomitant with DMH and white ginseng after DMH treatment inhibited the induction of DMH-induced ACF (aberrant crypt foci), which is preneoplastic lesion of colon cancer, in the colon of rats (18). The present investigation was carried out to evaluate the modifying effects of ginseng on chemical carcinogenesis in three different models in rats.

Experiment 1 was performed to investigate potential influence of white and red ginseng on rat hepatocarcinogenesis in a medium-term liver bioassay system, developed by Ito et al. (13). This model allows rapid screening of promoting or inhibitory activity of chemicals using a putative preneoplastic lesion, the glutathione S-transferase placental form (GST-P)-positive focus as the end point. In experiment 2, the dose dependence for red ginseng effects on rat hepatocarcinogenesis using the Ito test was further studied, because its possible promoting effects were observed in experiment 1. Experiment 3 was planned for the potential influence of red ginseng on initiation phase of rat hepatocarcinogenesis by using an initiation bioassay system (14) .

For chemoprevention, it is important to gain data on modifying effects in all major organs. In experiment 4, the effects of white and red ginsengs on tumor development were therefore investigated using a medium-term multi-organ carcinogenesis bioassay, the DMBDD model, already documented to be a useful tool for detection of carcinogens or chemopreventive agents in various organs in a relatively short period (15,16,17).

Materials and Methods

Animals

A total of 331 male five-week-old F344/DuCrj rats (Charles River, Hino, Shiga, Japan) were

housed per five to a plastic cage with hard wood chips for bedding, and fed a powdered diet CE2 (Clea Japan Inc., Osaka, Japan) and water *ad libitum*. The animals were maintained in an environmentally standard controlled. They were used in this study after a one-week acclimation period.

Chemicals

Diets containing ginseng were kindly provided by Wakunaga Pharmaceutical Co., Ltd. (Osaka, Japan). Diethylnitrosamine (DEN)(Wako Pure Chemical Industries Ltd., Osaka, Japan), N-methyl-N-nitrosourea (MNU) (Sigma Chemical Co., St.Louis, MO), N-butyl-N-(4-hydroxybutyl)-nitrosamine (BBN) (Tokyo Kasei Kogyo Co. Ltd., Tokyo, Japan), DMH(Tokyo Kasei Kogyo Co.Ltd., Tokyo, Japan) and dihydroxy-di-N-propylnitrosamine (DHPN) (Nacalai Tesqe Inc., Kyoto, Japan) were employed for initiation. 2-Acetylaminofluorene (2-AAF) was obtained from Tokyo Kasei Kogyo Co. Ltd.

Experimental protocols

Experiment 1 was performed to investigate the modifying effects of white and red ginseng on rat liver carcinogenesis using the Ito test. The experimental design is shown in Fig. 1. A total of 100 rats were divided randomly into 10 groups (15 rats each in groups 1~5, 5 rats each in groups 6~10). Animals in groups 1 to 5 were given a single intraperitoneal (i.p.) injection of DEN (200 mg/kg body wt.) dissolved in saline to initiate hepatocarcinogenesis. Groups 6-10 received saline alone. After 2 weeks, the rats in groups 1 and 6 were maintained on basal diet without ginseng. Animals in groups 2-5 and 7-10 received diet containing 1% white ginseng powder, 0.3% white ginseng extract, 1% red ginseng powder or 0.3% red ginseng extract, respectively, for the remaining experimental duration of 6 weeks. The level of the original ginseng in the 0.3% extract diet was equal to that in the 1% powdered diet. All rats were subjected to two-thirds partial hepatectmy at week 3. Surviving rats in each group were killed for examination at week 8. At autopsy, livers were excised and 3 mm-thick slices were cut with a razor blade and fixed in 10% buffered formalin for immunohistochemical demonstration of GST-P-positive foci.

Experiment 2 was performed to investigate the dose dependence of any effects of red ginseng on the post-initiation stage using the Ito test, partly for the purpose of confirmation of the results of experiment 1. A total of 78 rats were divided into five groups, all given a single i.p. injection of DEN. After 2 weeks, the rats in group 1 received basal diet, while groups 2,3,4 or 5 were given

diet containing 0.03, 0.1, 0.3 or 1% powdered red ginseng for the remaining experimental duration of 6 weeks, respectively. Surviving rats in each group were killed for examination at week 8, 1 hour after receiving an i.p. injection of 5-Bromo-2'-deoxyuridine (BrdU) (100 mg/kg body wt.). The livers were examined immunohistochemically for GST-P-positive foci, TGF-α-positive foci and BrdU-labelled cells.

Experiment 3 was planned to examine the modifying effects of red ginseng on the initiation stage using an initiation bioassay system (Takada et al., 1997). The experimental design is shown in Fig. 2. 78 rats were divided into five groups. All rats were given a single i.p. injection of DEN (20 mg/kg body wt.) dissolved in saline. They received diet containing powdered red ginseng at doses of 0, 0.03, 0.1, 0.3 or 1% from 6 days prior to DEN injection to one day after. All rats were fed 0.01% 2-AAF in powdered diet from weeks 2 to 4 and subjected to two-thirds partial hepatectomy at week 3. Throughout the experiment the animals had free access to food and water, and body weights were recorded once per week, along with food and water consumption. Surviving rats in each group were killed for examination at week 5. Immunohistochemical demonstration of GST-P-positive foci and TGF-α-positive foci was performed as described for experiment 1.

Experiment 4 was conducted to assess the modifying effects of white and red ginseng in a multi-organ carcinogenesis model. A total of 75 rats were divided randomly into 6 groups (20 rats each in groups 1-3, 5 rats each in groups 4-6) for treatment with test compounds. Those in groups 1 to 3 received combined treatments with a single i.p. injection of 100 mg/kg body weight of DEN, four i.p. injections of 20 mg/kg body weight of MNU, four s.c. injections of 40 mg/kg body weight of DMH, together with 0.05% BBN for 2 weeks, and then 0.1% DHPN for 2 weeks (both given in the drinking water), during the initial 4-week period for multiple organ initiation (DMBDD treatment). After this treatment with five carcinogens, all animals were given basal diet for 2 weeks and then these in groups 1 to 3 were administered basal diet, diet containing 1% powdered white ginseng or 1% powdered red ginseng for 30 weeks respectively. Groups 4 to 6 received vehicles without carcinogens in the first step followed by the respective test chemicals. At the beginning of week 37, all surviving animals were killed under ether anesthesia and subjected to complete necropsy. All major organs were excised, fixed in 10% buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin for histological examination. Livers fixed in 10% buffered formalin were examined after immunohistochemical demonstration of GST-P-positive foci.

Immunohistochemical staining

The avidin-biotin-peroxidase complex (ABC) method was used to demonstrate GST-P-positive foci. After deparaffinization, liver sections were treated sequentially with normal goat serum, anti-rabbit GST-P antibody (MBL Co., Ltd., Nagoya, Japan; 1:2000, overnight), biotin-labeled goat anti-rabbit IgG (1:400) for 1 hour and ABC. The numbers and the areas of GST-P positive foci (>0.2 mm in diameter) in the liver sections were measured using a color video image processor (IPAP, Sumika Technos Corp., Osaka, Japan). Immunohistochemical staining for BrdU was also performed using the ABC method. BrdU-labelled nuclei were counted under a microscope and labeling indices were calculated as percentages of labeled cells among ~5000 hepatocytes.

Results

Experiment 1

Final body weights were significantly increased in rats fed white ginseng without DEN initiation compared to the appropriate controls (data not shown). Numbers and areas of GST-P-positive foci in the livers of groups 1 to 5 in experiment 1 are summarized in Table 1. Values per unit area of liver section after DEN initiation were increased in rats treated with red ginseng powder, however without statistical significance. GST-P-positive foci were not seen in the non-initiated groups.

Experiment 2

Dietary administration of red ginseng did not affect the growth curves, final body weights and relative liver weights in the Ito test or the initiation bioassay system. During the period of the

Table 1. Numbers and Areas of Liver GST-P-positive Foci in the Livers of Rats in Experiment 1

Group	Treatment	Effective No. of rats	GST-P-positive Foci	
			No. / cm^2	Area(mm^2 / cm^2)
1	DEN → basal diet	14	2.48 ± 0.92[a]	0.19 ± 0.08
2	DEN → WG powder, 1%	12	2.52 ± 1.22	0.21 ± 0.11
3	DEN → WG extract, 0.3%	14	2.62 ± 1.28	0.24 ± 0.18
4	DEN → RG powder, 1%	14	3.48 ± 1.80	0.31 ± 0.21
5	DEN → RG extract, 0.3%	12	2.48 ± 1.02	0.29 ± 0.20

[a] Mean ± S.D.; WG, white ginseng; RG, red ginseng

Table 2. Numbers and Areas of Liver GST-P-positive Foci in the Livers of Rats in Experiment 2 (Ito test)

Group	Treatment	Effective No. of rats	GST-P-positive Foci	
			No. / cm^2	Area(mm^2 / cm^2)
1	DEN → basal diet	17	3.39± 1.65[a]	0.33± 0.22
2	DEN → RG, 0.03%	15	2.89± 1.32	0.31± 0.19
3	DEN → RG, 0.1%	14	3.55± 1.51	0.30± 0.15
4	DEN → RG, 0.3%	14	3.08± 1.16	0.30± 0.11
5	DEN → RG, 1%	15	3.82± 2.08	0.37± 0.22

[a]Mean± S.D.; RG, red ginseng

Table 3. Numbers and Areas of Liver GST-P-positive Foci and TGF-α-positive Foci in the Livers of Rats in Experiment 2(Initiation bioassay)

Group	Treatment	Effective No. of rats	GST-P-positive Foci	
			No. / cm^2	Area(mm^2 / cm^2)
1	DEN	13	17.11± 8.73[a]	1.84± 1.42
2	DEN+RG, 0.03%	14	15.32± 11.26	1.49± 1.50
3	DEN+RG, 0.1%	9	13.40± 6.25	1.42± 1.18
4	DEN+RG, 0.3%	10	15.49± 9.73	1.55± 1.08
5	DEN+RG, 1%	14	13.51± 6.73	1.35± 1.10

[a]Mean± S.D.; RG, red ginseng

experiment, no intergroup differences in daily food and water consumption were apparent in either case.

The numbers and areas of GST-P-positive foci in the rats given red ginseng at any dose after DEN were not significantly altered from the values for animals treated with DEN alone (Table 2). BrdU labeling indices in the livers have no effects being evident for the red ginseng treatment.

Experiment 3

Data for the numbers and areas of GST-P-positive foci in the liver in initiation bioassay are summarized in Table 3. The numbers and areas of GST-P-positive foci in the rats given red ginseng after DEN were not significantly affected as compared to the values for animals treated with DEN alone.

Experiment 4

Dietary administration of white and red ginseng did not affect the growth curves of rats,

Table 4. Histopathological findings in a variety of organs in Experiment 4

Organ and Findings	Group		
	1:Control (n=20)	2:WG (n=20)	3:RG (n=20)
Thyroids			
Follicular hyperplasia	19[a]	18	18
Follicular adenoma	12	9	10
Follicular carcinoma	4	9	9
Lungs			
Alveolar hyperplasia	20	20	20
Adenoma	11	13	15
Adenocarcinoma	8	12	9
Esophagus			
Squamous cell hyperplasia	19	20	20
Squamous cell papilloma	3	4	2
Squamous cell carcinoma	1	0	3
Forestomach			
Squamous cell hyperplasia	13	12	14
Squamous cell papilloma	6	2	5
Squamous cell carcinoma	2	1	1
Small intestine			
Adenoma	3	8	2
Adenocarcinoma	6	1[b]	4
Large intestine			
Adenoma	1	4	3
Adenocarcinoma	5	1	3
Liver			
Hepatocellular adenoma	2	0	2
Hepatocellular carcinoma	1	1	1
Kidneys			
Altered tubules	20	19	20
Adenoma	11	13	16
Transitional cell carcinoma	2	1	2
Nephroblastoma	19	15	20
Urinary bladder			
PN hyperplasia [c]	6	2	3
Transitional cell papilloma	1	2	1
Transitional cell carcinoma	2	1	1

[a] Number of animals bearing lesions
[b] Significantly different from group 1 at $p<0.05$
[c] PN, papillary or nodular
WG, white ginseng ; RG, red ginseng

mortalities and relative organ weights(liver, kidney, spleen). Histopathological findings for various organs in groups 1-3 are summarized in Table 4. The incidence of adenocarcinomas of

the small intestine was significantly reduced in the white ginseng treatment group and similar tendency was noted for large intestinal adenocarcinomas. However, total incidences of tumors of the small and large intestine did not differ among groups 1-3. Incidences of tumors and preneoplastic lesions in other organs did not significantly differ among the groups receiving the DMBDD treatment. No tumors or preneoplastic lesions were observed in any of the rats given ginseng without DMBDD-treatment (data not shown).

Discussion

The present study demonstrated that administration of white and/or red ginseng does not modify GST-P-positive foci development in the rat liver either promotion or initiation stages of rat hepatocarcinogenesis. These results indicate that ginseng lack chemopreventive effects for hepatocarcinogenesis in the rat. However, dietary administration of white ginseng did appear to suppress cancer development in the small intestine and colon, exerting inhibitory effects on the progression stage.

Ogiso *et al.* (19) reported that the degree of induction of GST-P-positive foci in the presently applied bioassay system (Ito test) corresponds with the induction of hepatocellular carcinomas as revealed by long-term *in vivo* studies. About 300 chemicals have already been analyzed and the efficacy of the system for detecting hepatocarcinogens, as well as chemopreventive agents has been well established (13).

Experiment 1 demonstrated that dietary administration of any ginseng does not inhibit GST-P-positive foci development in the medium-term liver bioassay system. However, red ginseng powder slightly tended to increase the numbers and areas of GST-P-positive foci. Therefore, experiment 2 was performed but promotion was not confirmed. Furthermore, cell proliferation, which plays an important role in carcinogenesis was not influenced in terms of BrdU labeling, a specific measure of DNA synthesis and proliferation. However, Xiu-gan *et al.* reported that red ginseng extract was found to inhibit induction of liver γ-GT-positive foci and cancer development in rats when given shortly after oral administration of DEN (12). Whether variation in the administration route of carcinogens, i.e., i.g. and i.p., is important remaining unclear. It is possible that oral administration of red ginseng extract 1 hr after DEN suppressed absorbance of the carcinogen from the intestine and/or metabolism of in their experiment.

Dietary administration of white ginseng suppressed cancer development in the small intestine

and the colon in experiment 4. Recently, we have also shown that dietary administration of white ginseng for 8 weeks after DMH-treatment was effective at reducing development of ACF in the rat colon (18). Thus inhibitory effects of white ginseng on tumor development in the intestine might be expected on the stage of progression. On the other hand, red ginseng did not show the inhibitory effects on colon tumor development in this study as like in the previous short-term experiment, when applied after DMH treatment(18). Therefore, red ginseng may exert chemopreventive effects on colon carcinogenesis when applied at initiation phase (18).

Ginseng did not have promoting effects in any organs under the present experimental conditions, so that our results suggest possible application for chemoprevention of colon, since no obvious adverse effects were noted. Further study is needed concerning mechanisms of chemopreventive effects of ginseng on rat colon carcinogenesis.

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