INTRODUCTION

Colon cancer is one of the most common malignancies in many regions of the world (1). Also in Japan, the numbers of cases of colorectal cancer are increasing with the spread of Western dietary habits (2). The idea that this cancer might be a candidate for chemoprevention stems from epidemiological evidence that some factors in the diet may play important roles in its development, whereas others may reduce the risk (3, 4). In animal studies, repeated treatment with a carcinogen, 1,2-dimethylhydrazine (DMH), produces colon tumors in rodents (5, 6). Colon carcinogenesis models using DMH or the related azoxymethane, with putative preneoplastic aberrant crypt foci (ACF) as end-point marker lesions, have been used to assess the influence of modulatory factors (7, 8).

ACF are readily discernible morphological changes within the colonic mucosa of rodents that may contribute to the stepwise progression to colon cancer (9-11). Pretlow and co-workers (12) showed that these lesions are also present in the colonic mucosa of patients with colon cancers and suggested that they are putative precursor lesions from which adenomas and carcinomas may develop. The formation and growth of ACF are associated with induction of colon tumors in rats and are influenced by exposure to chemopreventive agents (13, 14). Natural compounds that inhibit ACF induced by colon carcinogens have proved to be protective against colon cancer in rodents (13, 15).

Ginseng is believed to have been used in medicinal preparations for about 2000 yr in Oriental countries. Several pharmacological activities have been reported for ginseng extracts or ginseng dammarance saponins (16-19). Each type of ginseng can be ingested in various forms, for example, as fresh slices, juice, extract (tincture or boiled extract), powder, tea, tablet, or capsule (20). Preventive effects of ginseng against cancer development have been observed for many of these, including fresh ginseng extract, white ginseng powder, and red ginseng products, but not fresh ginseng slices, fresh ginseng juice, and white ginseng tea (20).

Antitumor effects of ginseng have also been supported by animal studies (21-26). For example, a medium-term (8) model system revealed anti-carcinogenic activity of ginseng regarding pulmonary adenoma induction by benzo[a]pyrene in newborn mice (27). In humans, anticancer influence of ginseng has been documented (20, 28). Although ginseng was found in one study to have a non-organ-specific preventive effect against cancer (29), there are weak negative associations between ginseng intake and cancers of the female breast, uterine cervix, urinary bladder, and thyroid gland. It is also associated with decreased odds ratios for cancers of the lip, oral cavity, pharynx, esophagus, stomach, colorec-

Inhibition by Ginseng of Colon Carcinogenesis in Rats

The inhibitory effects of ginseng on the development of 1,2-dimethylhydrazine (DMH)-induced aberrant crypt foci (ACF) in the colon were investigated in rats. Male, 6-week-old rats were injected with DMH once a week for 4 weeks. Rats in Groups 1 and 2 were fed diets containing red and white ginseng, respectively, at a dose of 1% for 5 weeks, starting one week before the first treatment of DMH. Animals in Groups 3 and 4 received red or white ginseng for 8 weeks starting after DMH treatment. Group 5 served as a carcinogen control group. Numbers of ACF with at least four crypts were significantly reduced in the colon of Group 2 treated with red ginseng combined with DMH. Moreover, rats were injected with DMH 4 times at one-week intervals. They were also fed diets containing 1% red or white ginseng or the control diet throughout 30 days of the experiment. Treatment with red ginseng resulted in a significant decrease of 5-bromo-2-deoxyuridine labeling indices in colonic crypts comprising ACF. These findings suggest that dietary administration of red ginseng in combination with DMH suppresses colon carcinogenesis in rats, and the inhibition may be associated, in part, with inhibition of cell proliferation, acting on ACF in the colonic mucosa.

Key Words: Ginseng; 1,2-Dimethylhydrazine; Rat Colon Carcinogenesis; Aberrant Crypt Foci

Address for correspondence
Shoji Fukushima, M.D.
To whom requests for reprints should be addressed, at the Department of Pathology*, Osaka City University Medical School, Osaka, Japan

Osaka City University Medical School, 1-4-3 Asahi-machi, Abeno-ku, Osaka 545-8585, Japan
Tel: +81-6-6646-3735, Fax: +81-6-6646-3903
E-mail: fukuchan@med.osaka-cu.ac.jp

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In the present study, inhibitory effects of ginseng in a DMH-induced colon carcinogenesis model in rats were assessed, with attention concentrated on quantitative values for preneoplastic ACF and cellular proliferative status in terms of 5-bromo-2′-deoxyuridine (BrdU) labeling (30).

**MATERIALS AND METHODS**

### Animals

A total of 125 male five-week-old F-344 rats (Charles River, Hino, Shiga, Japan) were housed five per plastic cage with wood chips for bedding in an animal room under standard conditions. Body weight and food intake were measured weekly during the experiment. Diet and water were available ad libitum.

### Chemicals

DMH was purchased from Wako Pure Chemical Industries (Osaka, Japan). White ginseng and red ginseng powders were obtained from Wakunaga Pharmaceutical Co. Ltd. (Hiroshima, Japan), and each was administered at a concentration of 1% in CE-2 basal diet (Clea Japan, Osaka, Japan). Ginseng powders were from the roots of *Panax ginseng* C.A. Meyer cultivated in China.

### Experimental protocols

In Experiment 1, 65 five-week-old rats were divided into eight groups. Animals in Groups 1-5 (10 rats in each group) were given DMH (20 mg/kg body weight, subcutaneous) once a week for 4 weeks. Rats in Groups 6-8 (5 rats each group) were subcutaneously injected with 0.9% saline (vehicle) at the same time. Groups 1 and 2 also received diets containing red and white ginseng, respectively, for 5 weeks, starting one week before the first DMH or saline treatment. Groups 3 and 4 were similarly fed diets containing red and white ginseng after DMH treatment (Weeks 5-12). Group 5 served as a carcinogen-treated control. The animals of Groups 6 and 7 were fed a diet containing red and white ginseng without DMH, and Group 8 (the vehicle control) received the basal diet alone throughout the experiment period.

In Experiment 2, 60 five-week-old rats were divided into 6 groups of 10 rats each and injected with DMH at 20 mg/kg body wt, sc (Groups 1-3) or saline alone (Groups 4-6) 4 times at one-week intervals (Weeks 1-4). The rats were fed diets containing red ginseng (Groups 1 and 4) and white ginseng (Groups 2 and 5) or the control diet (Groups 3 and 6), respectively, for 30 days, starting one week before the first DMH or saline treatment.

Animals were sacrificed under ether anesthesia at week 12 (Experiment 1) and the second day after the last injection of DMH (Experiment 2), respectively. All 60 rats in Experiment 2 received a single injection of BrdU (Sigma Chemical, St. Louis, MO; 100 mg/kg body wt, ip) one hour before sacrifice. After the colon was stained with 0.2% methylene blue as described previously (30), ACF were examined under a stereomicroscope. After they were counted, the colons were examined for BrdU immunohistochemistry, as described earlier (31).

### Statistical Analysis

Statistical analyses were completed with Stat-View software on a Macintosh computer. The significance of differences between average values for groups was analyzed using Cochran’s two-tailed Student’s t-test.

### RESULTS

#### Experiment 1

All rats survived to the final sacrifice. The body weights of animals given DMH and red or white ginseng were smaller than those treated with DMH alone, and final average body weights of rats in Groups 1-3 were significantly lower than those in the control groups. Among the vehicle-treated animals, the relative liver weights in Group 7 were significantly lower than those in Group 8. Food consumption on a rat-per-cage basis was comparable among the groups.

### Table 1. Effects of Ginseng on DMH-Induced ACF Formation in the Colons of Rats: Experiment 1a,b

| Group No. | No. of rats | Treatment   | Total No. of ACF | 1 crypt | 2 crypts | 3 crypts | ≥4 crypts |
|-----------|-------------|-------------|------------------|---------|---------|---------|----------|
| 1         | 10          | DMH + WG    | 282.1 ± 58.5     | 97.7 ± 34.9 | 89.3 ± 21.7 | 55.1 ± 15.0 | 37.9 ± 24.8 |
| 2         | 10          | DMH + RG    | 177.5 ± 92.3*    | 56.2 ± 28.2* | 70.4 ± 36.5 | 32.9 ± 24.1* | 18.2 ± 10.0* |
| 3         | 10          | DMH + R1W   | 223.7 ± 45.4*    | 81.6 ± 33.5 | 76.7 ± 14.8 | 39.4 ± 6.3** | 25.0 ± 11.2 |
| 4         | 10          | DMH + R2G   | 290.3 ± 83.6     | 89.8 ± 24.5 | 93.2 ± 27.5 | 64.3 ± 42.7 | 45.8 ± 31.3 |
| 5         | 10          | DMH         | 305.5 ± 56.0     | 115.5 ± 42.9 | 91.1 ± 20.8 | 61.7 ± 13.2 | 37.2 ± 13.8 |

DMH, 1,2-dimethylhydrazine; WG, white ginseng; RG, red ginseng; ACF, aberrant crypt foci.* Values are means ± SD; n: number of rats; **: Statistical significance is as follows: *, *p < 0.01; **, p > 0.001 vs. Group 5.
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Table 1. Summary of DMH-Induced ACF Formation in the Colons of Rats Fed the Control and Experimental Diets (Groups 1-5).

| Group No. | Treatment     | No. of rats | Total No. of ACF | No. of Foci Containing |
|-----------|---------------|-------------|------------------|------------------------|
| 1         | DMH + WG      | 10          | 111.0 ± 22.9     | 101.4 ± 23.1          | 8.2 ± 4.4              | 0.3 ± 0.5               | 0.1 ± 0.3               |
| 2         | DMH + RG      | 10          | 63.2 ± 19.3*     | 56.7 ± 16.3*          | 6.0 ± 4.2*             | 0.6 ± 0.7               | 0                      |
| 3         | DMH           | 10          | 169.6 ± 80.2     | 149.5 ± 75.2          | 13 ± 5.5               | 0.5 ± 0.7               | 0.1 ± 0.3               |

DMH, 1,2-dimethylhydrazine; WG, white ginseng; RG, red ginseng; ACF, aberrant crypt foci. *: Values are means ± SD; #: Statistical significance is as follows; *, p < 0.01 vs. Group 3.

Table 2. Effects of Ginseng on DMH-Induced ACF Formation in the Colons of Rats: Experiment 2a,b

| Group No. | Treatment     | No. of rats | Total No. of ACF | No. of Foci Containing |
|-----------|---------------|-------------|------------------|------------------------|
| 1         | DMH + WG      | 10          | 111.0 ± 22.9     | 101.4 ± 23.1          | 8.2 ± 4.4              | 0.3 ± 0.5               | 0.1 ± 0.3               |
| 2         | DMH + RG      | 10          | 63.2 ± 19.3*     | 56.7 ± 16.3*          | 6.0 ± 4.2*             | 0.6 ± 0.7               | 0                      |
| 3         | DMH           | 10          | 169.6 ± 80.2     | 149.5 ± 75.2          | 13 ± 5.5               | 0.5 ± 0.7               | 0.1 ± 0.3               |

DMH, 1,2-dimethylhydrazine; WG, white ginseng; RG, red ginseng; ACF, aberrant crypt foci. a: Values are means ± SD; b: Statistical significance is as follows; *, p < 0.01 vs. Group 3.

Experiment 2

Exposure to red ginseng (Group 2) relative to the control (Group 3) significantly reduced the formation of ACF (Table 2). Rats administered saline (Groups 4-6) had no ACF in the colon (data not shown). Crypts containing ACF had more BrdU-labeled cells than normal-appearing crypts. Crypts containing ACF in animals fed red ginseng had fewer BrdU-labeled cells than those of animals fed the basal diet. As shown in Fig. 1, the BrdU labeling index was significantly suppressed within ACF in the animals (Group 2) fed the diet with red ginseng. The inhibition was evident along the length of the colon.

DISCUSSION

The present study was undertaken to evaluate red or white ginseng for potential inhibition of ACF formation in the colon, putative preneoplastic lesions (10-12). The results indicate that dietary red ginseng powder exposure during the initiation phase exerts significant inhibition potential. The colon tumor incidence in rats correlates best with numbers of large ACF (≥ 4 crypts/focus), which are more likely to persist, increase in size through multiplication (9, 11), and develop into tumors (14). Large ACF are also linked with colon cancer development from the viewpoint of dysplastic change (10, 11), altered proliferative pattern (11), K-ras mutations (32), and DNA adduct formation (33). Therefore, the finding of significant reduction of large ACF (≥ 4 crypts/focus) might point to an effect on tumor yield. Although this requires confirmation in a long-term experiment, the results provide support for human intervention trials of red ginseng as a cancer-chemopreventive agent.

Ginseng has potent cancer-chemopreventive effects in humans (20, 23, 24, 28). Epidemiological studies have shown that the odds ratios of ginseng consumers are decreased with all kinds of cancers in case-control studies (20, 28). Recently, a prospective study to evaluate the preventive effect of ginseng against lung cancer in humans also revealed a reduced risk with ginseng intake, adjusted for smoking, with no cancer death among 24 red ginseng consumers (29). In the present study, significant decrease of the number of large ACF was not observed in animals given white ginseng powder, although total colonic ACF were reduced (Experiment 1). Therefore, the present study indicated that white ginseng is not as effective as red ginseng at inhibiting DMH-induction of ACF development in the rat colon.

Concerning the effects of red ginseng, Yun and co-workers...
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