Chemopreventive Effect of Selenium-Enriched Japanese Radish Sprout against Breast Cancer Induced by 7,12-Dimethylbenz[a]anthracene in Rats

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YAMANOSHITA, O., ICHIHARA, S., HAMA, H., ICHIHARA, G., CHIBA, M., KAMIJIMA, M., TAKEDA, I. and NAKAJIMA, T. Chemopreventive Effect of Selenium-Enriched Japanese Radish Sprout against Breast Cancer Induced by 7,12-Dimethylbenz[a]anthracene in Rats. Tohoku J. Exp. Med., 2007, 212 (2), 191-198 ——— Breast cancer is one of the major cancers in women, and dietary intake must be controlled to prevent it. Selenium (Se), especially Se compound in vegetables, is thought to be a promising chemopreventive dietary ingredient for preventing breast cancer. In this study, we developed Se-enriched Japanese radish sprout using a special Se-additional fertilizer, and identified the Se chemical forms. The newly developed Se-enriched sprout is produced within a week by the tank forming method, and the major chemical form was identified as Se-methylselenocysteine (MeSeCys) (80%). Then, the chemopreventive effects of the Se-enriched sprout were investigated using Sprague-Dawley female rats with mammary cancer, induced by a single oral dose of 10 mg or 14 mg of 7, 12-dimethylbenz[a]anthracene (DMBA). Mammary tumors were found in 11, 16 and 2 rats treated with DMBA and thereafter fed the basal (n = 34), sprout-added basal (n = 30) and Se-enriched sprout-added test diets (n = 30), respectively. The incidence of mammary tumors was significantly lower in the Se-enriched sprout-added test diet group (7%) than in the basal diet group (32%) or sprout-added basal diet group (53%). In contrast, no significant difference was detected in the numbers and incidence of the tumor between the basal diet group and Se-enriched sprout-added test diet group before DMBA-dosing. These results suggest that the diet supplement of Se-enriched sprout after DMBA-dosing provides a significant chemoprevention against chemical-induced mammary cancer. Thus, Se-enriched sprout may be a useful dietary ingredient for preventing breast cancer. ——— selenium; chemoprevention; 7,12-dimethylbenz[a]anthracene; breast cancer

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Breast cancer is one of the major cancers in women, and its incidence is still at a high level around the world (mortality rate, 15.6/100,000 in 2000) (Shibuya et al. 2002). Some intervention trials have been conducted to reduce the morbidity and the mortality of this cancer. One is by selenium (Se)-related regulatory functions in cell growth, cell survival and transformation, which is known to be embraced as a viable option (Greeder and Milner 1980; Milner and Hsu 1981; Medina et al. 1983; Medina and Shepherd 1984; Shamberger 1985; Borek et al. 1986). Although Se is an essential trace element existing in both prokaryotic and eukaryotic cells, it also has cytotoxicity with the lowest adverse effect of 0.023 mg/kg/day, which is about 20-fold the daily intake (Yang et al. 1983). Therefore, it is also possible that supplement abuse of Se results in increased body burden, and predisposes individuals toward toxicity.

Se exists in several chemical forms. Of many Se compounds, an organic Se (such as selenomethionine) is known to be more efficient than an inorganic form to prevent cancer development (Ip 1988). Further, the plant form Se, for example, the Se included in broccoli whose major chemical form is Se-methylselenocysteine (MeSeCys), was more effective in inhibiting colon carcinogenesis than an equivalent amount of inorganic Se or selenomethionine in rat (Finley and Davis 2001). In addition, Se-enriched garlic, whose major chemical form is γ-glutamyl-Se-methylselenocysteine, suppressed the development of 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary adenocarcinomas in rats more effectively than Se-enriched yeast, the chemical form of which was selenomethionine (Ip et al. 2000). Thus, Se compounds included in plants may be one of the most effective ways of inhibiting carcinogenesis.

In general, it is difficult to produce high levels of Se-containing vegetables with tank farming as well as in soil, because of the lower Se absorption by the vegetables. For example, the Se concentration of Se-enriched broccoli sprout with tank farming using selenate as one of the inorganic forms of selenium was only 60 ppm per dry weight (Finley et al. 2001). We developed a new method to produce highly Se-contained sprout, that is, Se-enriched Japanese radish sprout (Se-enriched sprout) with tank farming, using a special Se-additional fertilizer (Japan Patent No. 2969128). When using this fertilizer, Se concentration in the vegetable sprout was 10-fold higher than that of broccoli (see Materials and Methods). In this study, we clarified the selenium chemical form of Se-enriched sprout and its chemopreventive effect on mammary cancer induced by DMBA.

**MATERIALS AND METHODS**

**Production of Se-enriched sprout and test diets**

Japanese radish sprout (*Raphanus sativus*) was seeded in flat containers 3 inches deep together with growth fertilizer. After sprouting, the fertilizer in one container was changed to Se-added fertilizer (Japan Patent No. 2969128), while another flat container continued to be cultivated with the initial fertilizer. When the sprouts developed to 10 cm in about 7 days, they were harvested, immediately frozen and lyophilized. The high Se-enriched sprout was analyzed as containing over 600 ppm of Se/g of dry weight. Two kinds of test diet, Se-enriched sprout-added test diet and sprout-added basal diet, were prepared to add the respective lyophilized sprout to commercial rodent chow (basal diet) adjusted according to the National Research Council guideline. The final concentration of the sprouts in each diet was 2.7% (w/w). Se concentration in Se-enriched sprout-added test diet was 8.8 ppm, and that in sprout-added basal and basal diets was under 1 ppm.

**Identification of Se chemical form in Se-enriched sprout by high-performance liquid chromatography (HPLC)-inductive coupled plasma mass spectrometry (ICPMS)**

Approximately 0.1 g Se-enriched plant was mixed with 1 ml of distilled water, and then homogenized with a Teflon homogenizer. The homogenate was centrifuged at 8,000 × g for 10 min at room temperature. After passing through a 0.22 μm membrane filter, the filtrate was subjected to HPLC for identification of the chemical forms of Se in the sprout. HPLC conditions were as follows: column, Shodex Asahipak GS-320HQ (Showa Denko, Tokyo) for gel filtration or Hamilton PRP X-100 (Hamilton, NV, USA) for anion exchange; mobile phase, 50 mM ammonium acetate (pH 6.5); elution rate, 0.5 ml/
min (gel filtration) or 0.6 ml/min (anion exchange) at room temperature; and injection volume, 20 μl of filtrate sample. The elution was directly monitored with ICPMS (Agilent 7500, Yokogawa Analytical Systems, Tokyo) at ion intensities of 77 and 82 m/z. Selenate, selenite, selenomethionine (Wako, Osaka) and MeSeCys (Acros Organics, Geel, Belgium) were used as standards.

Animal treatments
This study was conducted according to the Guidelines for Animal Experiments of the Shinshu University Animal Center. Virgin female rats of Sprague-Dawley strain at 3 weeks of age (n = 180) were purchased from Japan SLC (Hamamatsu) and housed individually in stainless steel wire-bottomed cages in a room with controlled temperature, light and humidity.

Test diet administration before DMBA-dosing
Rats (four-weeks-old, n = 71) were randomly assigned to two groups (basal diet group and Se-enriched sprout-added test diet group). All rats were fed their respective test diet for 3 weeks before treatment with vehicle or DMBA. At 50 days of age, five rats in each group were treated with a single oral dose of 1 ml of sesame oil/rat by gavage, and the remaining rats were given a single oral dose of 10 mg DMBA/ml sesame oil/rats by gavage. All rats were weighed and palpated once a week to investigate for the presence of tumors.

After treatment with vehicle or DMBA, all rats were fed a basal diet for 28 weeks, and then sacrificed after being anesthetized with ether. Tumor parts of mammary glands were immediately removed and fixed with 10% neutralized formalin for histological examination.

Test diet administration after DMBA-dosing
Rats aged 50 days (n = 109) were randomly assigned to three groups (basal diet group, sprout-added basal diet group and Se-enriched sprout-added test diet group). Five rats in each group were treated with a single oral dose of 1 ml of sesame oil/rat by gavage, and the remaining rats were treated with a single oral dose of 14 mg DMBA (Tokyo Kasei Kogyo, Tokyo)/ml sesame oil/rats by gavage. All rats were weighed and palpated once a week to investigate for the presence of tumors. In this experiment, DMBA dose was changed from 10 mg to 14 mg/rat, because the rat surveillance was shortened.

After treatment with vehicle or DMBA, all rats were fed their respective test diet for 13 weeks, and then sacrificed after being anesthetized with ether. Tumor parts of mammary glands were immediately removed and fixed with 10% neutralized formalin for histological examination.

Statistical analysis
The percentage of mammary cancers determined by histopathology was compared among groups using χ² tests. One-way analysis of variance was conducted for comparison in terms of tumor multiplicity, weight and size among groups, followed by Tukey’s Multiple range test. Probability levels < 0.05 were used as a criterion for significance.

RESULTS
Chemical form of Se in Se-enriched sprout
Fig. 1 shows typical chromatograms of Se-enriched sprout on HPLC-ICPMS. There were a few peaks on the chromatograms; the largest one was identified as Se-methylselenocysteine (MeSeCys) using gel filtration (A) and anion exchange columns (B), and the amounts were about 80%. One of the two small peaks was identified as selenite, but the other was not identified.

Body weights and food consumption
Change in the mean body weights of rats with several kinds of test diet administrations before DMBA-dosing are shown in Fig. 2A. No significant differences among the four groups (basal diet and Se-enriched sprout-added test diet before vehicle dosing, basal diet and Se-enriched sprout-added test diet before DMBA dosing) were observed in body weight or food consumption (data not shown).

Changes in the mean body weights of rats with several kinds of test diet given after DMBA-dosing are shown in Fig. 2B. Retardation of increase in the body weight was seen in rats fed
Se-enriched sprout-added test diet, regardless of DMBA-dosing, from 3 weeks. This retardation might have been partially caused by the selenium included in the diets. No macroscopic abnormality, however, was observed in rats fed these diets. No difference was observed in food consumption among the six investigated groups (data not shown).

Histopathological finding of mammary cancer
Histopathologically, there was marked epithelial proliferation in the mammary tumor tissue of all rats, resulting in a fused glandular pattern (Fig. 3A, B and C), which was consistent with the diagnosis of low-grade ductal carcinoma (adenocarcinoma) of the rat breast. Thus, although the DMBA induced mammary adenocarcinoma, the Se-enriched sprout-added test diet did not appear to influence the nature of the mammary cancer.

Effect of sprout and Se-enrich sprout on DMBA-induced mammary cancer (Test diet administration before DMBA-dosing)
No tumor was observed in rats treated with the vehicle and then fed the basal and Se-enriched sprout-added test diet. In contrast, mammary tumors were found in 20 and 15 rats fed the basal- and Se-enriched sprout-added test diets, respectively, before DMBA-dosing (Table 1). Thus, no significant difference was seen in the numbers and incidence of the tumor between the basal diet group (65%) and Se-enriched sprout-added test diet group (50%). These results suggest that 3 weeks administration of Se-enriched sprout before DMBA-dosing does not affect the number of cancers per rat and the incidence. We also measured the weight of cancer tissues, but no difference was observed among groups (Table 1).

Fig. 1. Chromatograms of water extracted Se compounds on HPLC-ICPMS. A: gel filtration column (Shodex Asahipak GS-320HQ). B: Anion exchange column (Hamilton PRP X-100). MeSeCys, Selenomethionine; SeMet, Selenomethylselenocysteine; Std., standard.
basal diet group or sprout-added basal diet group. These results suggest that the sprout itself does not affect the incidence but that the Se-enriched sprout significantly reduces it. However, neither the sprout itself nor the Se-enriched sprout influenced the number of cancers per rat. We also measured the weight and size of cancer tissues, but no difference was found among groups (Table 2).

**DISCUSSION**

Of the many Se compounds involved in plants, MeSeCys is thought to be one of the most outstanding for its chemoprevention of chemical carcinogen-induced mammary cancer in rat model (Ip et al. 1996; Lu et al. 1996). The major Se form in the Se-enriched sprout used in this study was MeSeCys (80%), which clearly inhibited the
DMBA-induced mammary tumorigenesis in rats. Important point may be that chemoprevention effect is better achieved by administering Se-enriched sprout after, not before, the DMBA-dosing.

Several kinds of anticancer substances have been developed to date using Se-enriched vegetables. When the major chemical form of γ-glutamyl-Se-methylselenocysteine (73%), namely, Se-enriched garlic, was added to the diet (3 ppm as Se), the incidence of tumors treated either by DMBA or MNU decreased from 83% - 93% to 33% - 47% (Ip et al. 1996, 2000; Lu et al. 1996). Moreover, when Se-enriched broccoli, a major chemical form (45%) of MeSeCys, was added (as final 3 ppm), the tumor incidence fell from 90% to 37% (Finley et al. 2001). The pure chemical form of γ-glutamyl-Se-methylselenocysteine and MeSeCys also decreased the incidence in the same manner as their plant form of selenium (Dong et al. 2001), suggesting that the chemoprevention potential of these two Se compounds is very similar. In the present study, the Se-enriched Japanese radish sprout we developed completely inhibited DMBA-induced mammary tumor. However, we used 8.8 ppm Se, which is considerably higher than the concentration (3 ppm) employed in the past experiments using garlic or broccoli (Ip et al. 2000; Finley et al. 2001). Therefore, the chemoprevention potential of Se-enriched Japanese radish sprout could not be directly compared to that of these vegetables. Since the MeSeCys content (80%) of the Se-enriched sprout we developed is higher than that (45%) of Se-enriched broccoli, the Se-enriched Japanese radish sprout may have higher chemopreventive effect compared to the broccoli for the mammary cancer induced by DMBA, when the same Se dose is used.

Many Se-enriched plants and yeast have been developed, and major selenocompounds containing these biological materials are as follows: garlic, γ-glutamyl-Se-methylselenocysteine; onion, MeSeCys; broccoli sprouts, MeSeCys; leeks, MeSeCys; and yeast, SeMet (Cai et al. 1995; Guo and Wu 1998; Kotrebai et al. 1999; Ip et al. 2000; Finley et al. 2001; Whanger 2002). Contents of MeSeCys are as follows: grassland legume, 10-13%; garlic, 3%; onion, 42-55%; broccoli sprout, 45%; leeks, 35-50%; and yeast,
Compared to these several kinds of biological materials developed earlier, the percentage of MeSeCys in the Se-enriched sprout we developed was the highest. In this regard, the Se-enriched sprout developed in the present study is superior to other plants such as garlic and broccoli sprout.

Se-enriched sprout is easy to produce by tank farming without soil or peat moss within about one week. Although Se-enriched florets of broccoli, garlic or wild leeks were produced in soil or peat moss, it takes several weeks, and their Se concentrations of Se are lower (Ip et al. 1992; Whanger et al. 2000; Finley et al. 2001) than the Se-enriched Japanese radish sprout we produced.

Thus, the tank farming method to produce Se-enriched plant using a special fertilizer (Japan Patent No. 2969128) is dominant compared to that fertilized in soil.

In conclusion, the Se-enriched sprout we developed clearly inhibited DMBA-induced tumorigenesis, suggesting that this sprout is a promising way to decrease mammary tumor incidence. However, the Se level used in the present study may have been too high, and possibly toxic. Further study using a lower Se concentration may be needed to identify the true chemopreventive effect of the Se-enriched sprout.

### Table 1. Effects of dietary Se-enriched sprout against mammary tumors when the sprout was fed before DMBA dosing.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of rats</th>
<th>Dose</th>
<th>Total Tumor number</th>
<th>Tumor Multiplicity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Tumor Incidence (%)</th>
<th>Tumor weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal diet with vehicle</td>
<td>5</td>
<td>vehicle</td>
<td>0</td>
<td>0/5 (0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Se-enriched sprout-added test diet with vehicle</td>
<td>5</td>
<td>vehicle</td>
<td>0</td>
<td>0/5 (0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Basal diet with DMBA</td>
<td>31</td>
<td>DMBA</td>
<td>59</td>
<td>2.95 ± 2.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20/31 (65)</td>
<td>0.86 ± 0.96</td>
</tr>
<tr>
<td>Se-enriched sprout-added test diet with DMBA</td>
<td>30</td>
<td>DMBA</td>
<td>36</td>
<td>2.40 ± 1.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15/30 (50)</td>
<td>1.60 ± 3.30</td>
</tr>
</tbody>
</table>

<sup>a</sup>Tumor multiplicity indicates the number of cancer tissues per rat with mammary cancer.  <sup>b</sup>Mean ± s.d.

### Table 2. Effects of dietary Se-enriched sprout against mammary tumors when the sprout was fed after DMBA dosing.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of rats</th>
<th>Dose</th>
<th>Total Tumor number</th>
<th>Tumor Multiplicity&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Tumor Incidence (%)</th>
<th>Tumor weight (g)</th>
<th>Tumor diameter (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal diet with vehicle</td>
<td>5</td>
<td>vehicle</td>
<td>0</td>
<td>0/5 (0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sprout-added basal diet with vehicle</td>
<td>5</td>
<td>vehicle</td>
<td>0</td>
<td>0/5 (0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Se-enriched sprout-added test diet with vehicle</td>
<td>5</td>
<td>vehicle</td>
<td>0</td>
<td>0/5 (0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Basal diet with DMBA</td>
<td>34</td>
<td>DMBA</td>
<td>14</td>
<td>1.27 ± 0.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11/34 (32)</td>
<td>3 ± 7.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.5 ± 1.2&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Sprout-added basal diet with DMBA</td>
<td>30</td>
<td>DMBA</td>
<td>20</td>
<td>1.25 ± 0.78&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16/30 (53)</td>
<td>1.5 ± 1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.6 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Se-enriched sprout-added test diet with DMBA</td>
<td>30</td>
<td>DMBA</td>
<td>2</td>
<td>1/2 (7)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Tumor multiplicity indicates the number of cancer tissues per rat with mammary cancer.  <sup>b</sup>Mean ± s.d.  
<sup>c</sup>Mean.  <sup>d</sup>Significantly different from basal-diet with DMBA groups and sprout-added basal diet with DMBA group, p < 0.05.
Acknowledgments

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References


