Evaluation of Nutritional Availability and Anti-Tumor Activity of Selenium Contained in Selenium-Enriched Kaiware Radish Sprouts

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We estimated the nutritional availability of selenium (Se) in Se-enriched Kaiware radish sprouts (SeRS) by the tissue Se deposition and glutathione peroxidase (GPX) activity of rats administered the sprouts, and examined the effect of SeRS on the formation of aberrant crypt foci (ACF) in the colon of mice administered 1,2-dimethylhydrazine (DMH) to evaluate anti-tumor activity. Male weanling Wistar rats were divided into seven groups and fed a Se-deficient basal diet or the basal diet supplemented with 0.05, 0.10, or 0.15 g/g of Se as sodium selenite or SeRS for 28 d. Supplementation with Se dose-dependently increased serum and liver Se concentrations and GPX activities, and the selenite-supplemented groups showed a higher increase than the SeRS-supplemented groups. The nutritional availability of Se in SeRS was estimated to be 33 or 64% by slope ratio analysis. Male 4-week-old A/J mice were divided into seven groups and fed a low Se basal diet or the basal diet supplemented with selenite, SeRS, or selenite + non-Se-enriched radish sprouts (NonSeRS) at a level of 0.1 or 2.0 µg Se/g for 9 weeks. After 1 week of feeding, all mice were given six subcutaneous injections of DMH (20 mg/kg) at 1-week intervals. The average number of ACF formed in the colon of mice fed the basal diet was 4.3. At a supplementation level of 0.1 µg Se/g, only SeRS significantly inhibited ACF formation. At a supplementation level of 2.0 µg Se/g, both selenite and SeRS significantly inhibited ACF formation. The addition of NonSeRS to the selenite-supplemented diets tended to inhibit ACF formation, but this was not statistically significant. These results indicate that SeRS shows lower nutritional availability but higher anti-tumor activity than selenite.

Key words: selenium; selenium-enriched sprouts; nutritional availability; cancer prevention; aberrant crypt foci

Selenium (Se) is an essential trace element in human and animal nutrition, and it plays several important roles in the form of selenoproteins, including the families of glutathione peroxidases (GPXs), deiodinases and thioredoxin reductases.1) The average Se intake in the Japanese population is about 100 µg/d/capita.2–4) This estimated value is obviously higher than the Recommended Dietary Allowance (RDA) of Se for adults, but since foods with high Se content are limited to particular food groups such as fish, eggs, meats, and US hard wheat,4) a severely unbalanced diet may cause low Se status. It has been pointed out that vegetarians and vegans are most at risk from low Se intakes.5) Worldwide, there are some low Se areas, such as New Zealand and Finland.6) To prevent low Se status, the preparation of various types of high Se food is useful to increase daily Se intake.7)

The utilization of dietary minerals including Se is the net result of several physiological and metabolic processes that convert a portion of ingested minerals to certain metabolically critical forms that are necessary for normal physiological function. As for mineral nutrition, it is necessary to show the extent of biological utilization of dietary minerals in their critical or functional forms quantitatively. The quantitative description of biological utilization of dietary minerals has come to be called their bioavailability.8) More strictly, the term bioavailability must be replaced by nutritional availability, since there exists the impression that bioavailability includes not only nutritional but also pharmacological effects.

Abbreviations: Se, selenium; SeRS, selenium-enriched Kaiware radish sprouts; NonSeRS, non selenium-enriched Kaiware radish sprouts; GPX, glutathione peroxidase; ACF, aberrant crypt foci; MeSec, Se-methylselenocysteine; ITC, isothiocyanate; DMH, 1,2-dimethylhydrazine; RDA, Recommended Dietary Allowance; HPLC, high performance liquid chromatography; ICPMS, inductively coupled plasma mass spectrometry; LOAEL, lowest observed adverse effect level; UL, tolerable upper intake level

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logical activities. The nutritional availability of dietary Se varies with the chemical species of Se in foods. Since the compositions of Se species in Se-enriched foods are diverse, their nutritional availability is thought to vary with the kind of foods. Accordingly, Se-enriched foods should be evaluated for its nutritional availability.

Besides nutritional roles, Se is thought to be associated with cancer prevention, judging by the results of epidemiological studies. In particular, a recent finding, that overall cancer morbidity and mortality were nearly 50% lower with daily supplementation with Se at a level of 200 μg/d, is of great interest. The anti-tumor activity of Se has also been confirmed in numerous animal experiments and a monomethylated Se metabolite is critical in Se chemoprevention. The metabolic conversion rate of monomethylated selenoamino acids such as MeSec and γ-glutamyl-SMeSec to the monomethylated Se metabolite is probably higher than that of selenite, selenate, or selenocysteine. These monomethylated selenoamino acids have been identified in several Se-enriched vegetables. The anti-tumor activities of these Se-enriched vegetables have also been evaluated, and have been found to be higher than that of selenite. Hence, the applicability of Se-enriched vegetables to cancer prevention is to be expected.

In previous studies, we prepared Se-enriched sprouts of various plant species including Kaiware radish and identified the main chemical species of Se in these Se-enriched sprouts as MeSec. The present study, we estimated the nutritional availability of Se in Se-enriched Kaiware radish sprouts (SeRS) by tissue Se deposition and GPX activity, and also evaluated anti-tumor activity of SeRS.

In animal experiments to examine the anti-tumor activity of natural products, various chemical carcinogens have been used to induce tumors in liver, colon, and mammary gland. Among these chemicals, 1,2-dimethylhydrazine (DMH) has often been used to induce colon cancer. Since DMH injected is excreted in the bile after conversion to an active metabolite in various organs, the colon is most exposed to the active carcinogenic metabolite. Consequently, the active metabolite causes alkylation of DNA mainly in the colon, and induces colon cancer specifically.

On the other hand, it has been proposed that aberrant crypt foci (ACF) are preneoplastic lesions and that those with a crypt multiplicity of more than 4 continue growing to tumors. Since the experimental period for formation of ACF is short and the identification of ACF formation is done readily, ACF has been used as an index of precancerous lesions in the colon. Hence we therefore scored the number of ACF with a crypt multiplicity of more than 4 as an index for the risk of colon cancer in the present study.

<table>
<thead>
<tr>
<th>Table 1. Composition of Basal Se-Deficient Diet Used in Experiment 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredients</td>
</tr>
<tr>
<td>Torula yeast</td>
</tr>
<tr>
<td>Sucrose</td>
</tr>
<tr>
<td>Soybean oil</td>
</tr>
<tr>
<td>AIN93G salt mixture</td>
</tr>
<tr>
<td>AIN93G vitamin mixture</td>
</tr>
<tr>
<td>Choline bitartrate</td>
</tr>
<tr>
<td>DL-Methionine</td>
</tr>
</tbody>
</table>

KR yeast kindly supplied by Kōhjin (Tokyo). Crude protein content was 51.2%.

Except for sodium selenate.

### Materials and Methods

**Preparation of SeRS.** Seeds of Kaiware radish (a type of Japanese white radish (daikon), the sprouts of which are eaten (scientific name, *Raphanus sativus*)) were purchased from a local retail shop in Osaka, Japan. SeRS were prepared by hydroponics using 10 μg Se/ml of sodium selenite solution, as described previously. Non-Se-enriched radish sprouts (NonSeRS) were also prepared using deionized water. SeRS and NonSeRS were freeze-dried and milled. The Se contents of the SeRS and NonSeRS were 110 and 0.03 μg/g dry weight respectively.

**Animal feeding.** The experimental protocol was reviewed and approved by the Animal Ethics Committee of Kansai Medical University and followed the “Guide for the Care and Use of Experimental Animals” of the Prime Minister’s Office of Japan. Experimental animals were fed in a room under a controlled 12 h light (8:00 to 20:00) and dark cycle at a temperature of 22 to 24°C respectively and a humidity of 60%. The animals were given experimental diets and deionized water ad libitum during the entire experimental period.

In experiment 1, 42 male weanling Wistar rats were divided into seven groups and fed a Torula yeast-based Se-deficient basal diet or the basal diet supplemented with 0.05, 0.10, or 0.15 μg/g of Se as sodium selenite or dried powder of the SeRS for 28 d. The composition of the basal Se-deficient diet is shown in Table 1. Analysis showed the basal Se-deficient diet to contain less than 0.01 μg Se/g. After feeding for 28 d, the rats were anesthetized with diethyl ether, blood was collected from the aorta abdominalis, and the liver was excised, washed, blotted, and weighed.

In experiment 2, 84 male 4-week-old A/J mice were divided into seven groups and fed a casein-based low Se basal diet or the basal diet supplemented with selenite, dried powder of the SeRS, or selenite + dried powder of NonSeRS at a level of 0.1 or 2.0 μg Se/g for 9 weeks. The supplementary level of NonSeRS was equal to that of SeRS; when the supplementary Se levels were 0.1 or 2.0 μg/g, supplementary amounts of both sprouts were 0.91 or 18.2 mg/g respectively. The composition of the
basal low Se diet is shown in Table 2. Since α-linolenic acid inhibits the development of mammary gland and colon cancer induced by DMH, 25) we used corn oil, which contains a lower level of this polyunsaturated fatty acid than soybean oil. Analysis showed the low Se basal diet to contain 0.035 μg Se/g. After 1 week of feeding, all mice were given six subcutaneous injections at 1-week intervals of saline containing DMH (20 mg/kg body weight). After feeding for 9 weeks, the mice were anesthetized with diethyl ether, blood was collected by heart puncture, the liver was removed, washed with saline, blotted, and weighed, and the colon was removed, opened longitudinally, washed with saline, and fixed flat between paper towels in a Formalin solution. An operator who was unaware of the dietary treatment scored the fixed colon of mice was stained with 0.02% methylene blue for 3 min and then washed with saline. An operator who was unaware of the dietary treatment scored the number of ACF in the stained colon under a dissecting microscope. In the present study, we scored the number of ACF with a crypt multiplicity of more than 4 as described above.

Table 2. Composition of Basal Low Se Diet Used in Experiment 2

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>20.0</td>
</tr>
<tr>
<td>α-Corn starch</td>
<td>13.2</td>
</tr>
<tr>
<td>β-Corn starch</td>
<td>39.75</td>
</tr>
<tr>
<td>Sucrose</td>
<td>10.0</td>
</tr>
<tr>
<td>Corn oil</td>
<td>7.0</td>
</tr>
<tr>
<td>AIN93G salt mixture *</td>
<td>3.5</td>
</tr>
<tr>
<td>AIN93G vitamin mixture</td>
<td>1.0</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.25</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5.0</td>
</tr>
<tr>
<td>l-Cystine</td>
<td>0.3</td>
</tr>
</tbody>
</table>

*Except for sodium selenate.

To determine Se, these diluted digests were directly nebulized to ICPMS and the ion intensity of 82Se was monitored.

Protein was measured by the method of Lowry et al., 30) with bovine serum albumin as a standard. In experiment 1, serum biochemical tests, including total protein, albumin, alanine aminotransferase, aspartate aminotransferase, total lipid, total cholesterol, urea nitrogen, and creatinine, were also performed by a commercial service (Japan Medical Laboratory, Osaka, Japan).

Analysis of ACF in colon of mice in experiment 2. The fixed colon of mice was stained with 0.02% methylene blue for 3 min and then washed with saline. An operator who was unaware of the dietary treatment scored the number of ACF in the stained colon under a dissecting microscope. In the present study, we scored the number of ACF with a crypt multiplicity of more than 4 as described above.

Assessment of nutritional availability of Se. In experiment 1, the nutritional availability of Se from SeRS was assessed using sodium selenite as reference Se. The deposition of Se and the increase in GPX activity in the liver and serum were used as responses to increasing amounts of dietary Se. Since the responses (R) to increasing amounts of dietary Se (X) were assumed to be described by the general equation $R = mX + k$, the relative nutritional availability of Se from SeRS was estimated by the slope-ratio technique, which compares the slope of dose-response plots to the slope observed for selenite Se. Nutritional availability was defined as $(\text{slope of SeRS})/(\text{slope of selenite}) \times 100$. 8)

Statistics. Experimental data were assessed by one-way analysis of variance. When the F value was
significant \((p < 0.05)\), the Tukey-Kramer multiple range test was performed to determine which pairs of the means were significantly different \((p < 0.05)\). These statistical tests were performed using a personal computer \(\text{eMac, Apple Computer, Cupertino, CA}\) with operating system Mac OS 9.2 and statistical program package StatView-J version 5.0 \(\text{(Abacus Concept, Berkeley, CA)}\).

**Results**

**Experiment 1**

During the entire feeding period of 28 d, no significant differences were observed in body weight or animal growth irrespective of dietary Se status. At the end of the experimental period, the mean \(\pm SE\) of the body weight \(\text{g}\) of the various groups were as follows: basal, \(269 \pm 9\); + 0.05 \(\mu g\) Se/g as selenite, \(271 \pm 5\); + 0.05 \(\mu g\) Se/g as SeRS, \(260 \pm 7\); + 0.10 \(\mu g\) Se/g as selenite, \(268 \pm 13\); + 0.10 \(\mu g\) Se/g as SeRS, \(265 \pm 10\), + 0.15 \(\mu g\) Se/g as selenite, \(270 \pm 3\); + 0.15 \(\mu g\) Se/g as SeRS, \(260 \pm 8\). Similarly, the effect of Se supplementation was least significant on liver weight and serum biochemistry \(\text{data not shown)}\).

The response of Se deposition and GPX activities in the liver and serum are summarized in Tables 3 and 4. Se deposition and GPX activities both increased gradually with increasing of supplementary levels of Se, regardless of source or of the tissue monitored. These responses did not tend to level off within the range of Se levels tested in the present study, but responses varied with Se source. Selenite Se led to a higher accumulation of Se and a higher elevation of GPX than did sprout Se. In particular, the four parameters in rats supplemented with selenite showed significantly higher values than those in rats supplemented with SeRS, at supplementary levels of 0.10 and 0.15 \(\mu g\) Se/g. This difference in the Se source was more remarkable in the liver than in the serum.

Table 5 summarizes the results of regression analysis between Supplementary Se Levels and Tissue Se Contents and GPX Activities in Experiment 1.
When the supplementary Se level was 0.1 g Se/g as selenite, 2.0 g Se/g as SeRS, 26.6 ± 0.5; + 0.1 µg Se/g as selenite and NonSeRS, 26.7 ± 0.5; + 2.0 µg Se/g as selenite, 26.8 ± 1.0; + 2.0 µg Se/g as SeRS, 25.9 ± 1.3; + 2.0 µg Se/g as selenite and NonSeRS, 26.3 ± 0.5.

Tables 7 and 8 summarize Se deposition and GPX activities in the liver and serum of the mice. The response patterns for the four parameters were similar. When the supplementary Se level was 0.1 µg/g, responses by selenite Se were higher than those of Se in the SeRS irrespective of supplementation with NonSeRS. On the other hand, when the supplementary level was 2.0 µg Se/g, the responses in mice supplemented with SeRS were higher than those in other mice; supplementation with high amounts of NonSeRS inhibited the elevation of Se deposition and GPX activities.

Table 9 shows the number of ACF formed in the colon of mice injected with DMH. The average number of ACF formed in the colon of mice fed the basal low Se diet was 4.3. Supplementation with Se to the basal diet showed an inhibitory effect on the formation of ACF, but the extent of inhibition varied with the Se source and the supplementary level. When the supplementary Se level was 0.1 µg/g, a significant inhibitory effect on the formation of ACF was observed only in mice supplemented with SeRS. On the other hand, when the supplementary level was 2.0 µg Se/g, both selenite and SeRS showed a significant inhibitory effect on the formation of ACF, but an increase in the supplementary level of SeRS to 2.0 µg Se/g did not cause a further decrease in ACF formation. The addition of NonSeRS to the selenite-supplemented diets tended to inhibit the formation of ACF, but this inhibition was not statistically significant (p > 0.05).

Table 7. Se Deposition of Mice in Experiment 2

<table>
<thead>
<tr>
<th>Source</th>
<th>Level (µg/g)</th>
<th>Supplemented sprouts</th>
<th>Se deposition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Liver (ng/g tissue)</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>—</td>
<td>151 ± 14a</td>
</tr>
<tr>
<td>Selenite</td>
<td>0.1</td>
<td>—</td>
<td>328 ± 50bc</td>
</tr>
<tr>
<td>SeRS</td>
<td>0.1</td>
<td>SeRS</td>
<td>240 ± 20ab</td>
</tr>
<tr>
<td>Selenite</td>
<td>0.1</td>
<td>NonSeRS</td>
<td>234 ± 18ab</td>
</tr>
<tr>
<td>Selenite</td>
<td>2.0</td>
<td>—</td>
<td>512 ± 36d</td>
</tr>
<tr>
<td>SeRS</td>
<td>2.0</td>
<td>SeRS</td>
<td>412 ± 23cd</td>
</tr>
<tr>
<td>Selenite</td>
<td>2.0</td>
<td>NonSeRS</td>
<td>327 ± 21bc</td>
</tr>
</tbody>
</table>

Values are the means ± SE (n = 12). Means not sharing a common superscript in the same column differ significantly at p < 0.05.

Table 8. GPX Activities of Mice in Experiment 2

<table>
<thead>
<tr>
<th>Source</th>
<th>Level (µg/g)</th>
<th>Supplemented sprouts</th>
<th>GPX activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Liver (unit/g protein)</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>—</td>
<td>229 ± 30a</td>
</tr>
<tr>
<td>Selenite</td>
<td>0.1</td>
<td>—</td>
<td>723 ± 84a</td>
</tr>
<tr>
<td>SeRS</td>
<td>0.1</td>
<td>SeRS</td>
<td>427 ± 35ab</td>
</tr>
<tr>
<td>Selenite</td>
<td>0.1</td>
<td>NonSeRS</td>
<td>820 ± 55a</td>
</tr>
<tr>
<td>Selenite</td>
<td>2.0</td>
<td>—</td>
<td>827 ± 63a</td>
</tr>
<tr>
<td>SeRS</td>
<td>2.0</td>
<td>SeRS</td>
<td>521 ± 37b</td>
</tr>
<tr>
<td>Selenite</td>
<td>2.0</td>
<td>NonSeRS</td>
<td>480 ± 45b</td>
</tr>
</tbody>
</table>

Values are the means ± SE (n = 12). Enzyme units expressed as µmol NADPH oxidized per min. Means not sharing a common superscript in the same column differ significantly at p < 0.05.
range of supplementation, regardless of the response measures employed for nutritional assessment. This was also confirmed in experiment 2 (Tables 7 and 8). Supplementation with SeRS gave a lower elevation of tissue Se deposition and GPX activities than selenite did in mice.

We have identified the main Se species in SeRS as MeSec.20) Since dietary Se must be metabolized to selenide before incorporation into selenoproteins,11 a lower elevation of GPX in tissues of rats or mice given SeRS indicates that demethylation of MeSec is negligible, but occurs to a certain extent in the tissues. The estimated availability varied according to which response data were used in the assessment; the availability values of the serum parameters were two times higher than the liver parameters. The molecular species differs between liver and serum GPX; while the former is called classical GPX (GPX1), the latter, called extracellular GPX (GPX3), is synthesized in the kidney and secreted into the plasma.31) Accordingly, the difference in responses, as between liver and serum might be caused by differences in demethylation ability between the liver and the kidney.

Supplementation with a high amount of NonSeRS inhibited the elevation of Se deposition and GPX activities caused by 2.0 \( \mu \)g Se/g of selenite in experiment 2. This indicates that components in radish sprouts lowered the nutritional availability of Se. Cruciferous vegetables, including Kaiware radish, contain several isothiocyanates (ITCs) as pungent taste substances, and the major ITC in Japanese white radish has been identified as 4-(methylthio)-3-butenyl isothiocyanate.32) the major ITC in Japanese white radish has been identified as 4-(methylthio)-3-butenyl isothiocyanate.32) Since dietary Se at high levels inhibits ACF formation in the colon and prevents colon cancer. At a supplementary level of 0.1 \( \mu \)g Se/g, only SeRS showed significant inhibition of the formation of ACF. This indicates that Se in SeRS has higher anti-tumor activity than selenite Se. Similar higher anti-tumor activity in Se-enriched vegetables has been reported for broccoli,19,36) garlic,37) and ramps38) at a dietary Se level of 1.0 \( \mu \)g/g or more. Since the main Se species in these Se-enriched vegetables, including SeRS, has been identified as MeSec,20,15–18,20) and a monomethylated Se metabolite is critical in Se chemoprevention,14) the higher anti-tumor activity in these Se-enriched vegetables is thought to be derived from MeSec.

Although an anti-tumor effect of Se has been found at a dietary level of 1.0 \( \mu \)g/g or more even in the case of Se-enriched vegetables in previous studies,19,36–38) SeRS inhibited ACF formation at a dietary level of 0.1 \( \mu \)g Se/g in the present study. The anti-tumor activity of SeRS is considered to be higher than that of other Se-enriched plant foods. Since about 90% of Se species in the SeRS used in the present study were identified as MeSec,20) the inhibitory effect caused by lower level of SeRS may be associated with the high ratio of MeSec in Se species of SeRS.

Another purpose of the present study was to evaluate the anti-tumor activity of SeRS. As described in Table 9, Se added to the low Se diet at a level of 2.0 \( \mu \)g/g inhibited the formation of ACF irrespective of the Se source. A similar inhibitory effect of high dietary Se (1.0 \( \mu \)g/g or more) on ACF formation has been reported for selenite,34) selenomethionine,35) high Se broccoli,19) and high Se broccoli sprouts.36) Hence, it is possible that dietary Se at high levels inhibits ACF formation in the colon and prevents colon cancer.

At a supplementary level of 0.1 \( \mu \)g Se/g, only SeRS showed significant inhibition of the formation of ACF. This indicates that Se in SeRS has higher anti-tumor activity than selenite Se. Similar higher anti-tumor activity in Se-enriched vegetables has been reported for broccoli,19,36) garlic,37) and ramps38) at a dietary Se level of 1.0 \( \mu \)g/g or more. Since the main Se species in these Se-enriched vegetables, including SeRS, has been identified as MeSec,20,15–18,20) and a monomethylated Se metabolite is critical in Se chemoprevention,14) the higher anti-tumor activity in these Se-enriched vegetables is thought to be derived from MeSec.

Although an anti-tumor effect of Se has been found at a dietary level of 1.0 \( \mu \)g/g or more even in the case of Se-enriched vegetables in previous studies,19,36–38) SeRS inhibited ACF formation at a dietary level of 0.1 \( \mu \)g Se/g in the present study. The anti-tumor activity of SeRS is considered to be higher than that of other Se-enriched plant foods. Since about 90% of Se species in the SeRS used in the present study were identified as MeSec,20) the inhibitory effect caused by lower level of SeRS may be associated with the high ratio of MeSec in Se species of SeRS.

On the other hand, the addition of NonSeRS to the selenite-supplemented diets tended to inhibit the formation of ACF. This leads to the possibility that NonSeRS also inhibits ACF formation. It has been reported that ITCs in cruciferous vegetables inhibit the development of several types of tumors.33) Because the Se concentration of SeRS (110 \( \mu \)g/g dry weight) used in the present study was lower than that in the high Se broccoli and broccoli sprouts used in previous reports,19,36) more Se-enriched vegetables were added to the diet to adjust the dietary Se level to 0.1 \( \mu \)g/g in the present study than in previous reports. Hence the ITC content in the diet supplemented with SeRS must have been higher than in the diets with high Se-broccoli or Se-broccoli sprouts at an equivalent Se level, and this high level of ITCs may have elevated the inhibitory effect of SeRS on ACF formation at the low supplementary level.
The present experimental results indicate that SeRS has lower nutritional availability but higher anti-tumor activity than selenite. This high anti-tumor activity of SeRS indicates the use of this high-Se plant food in the diet for cancer prevention. However, careful consideration is necessary as to the use of SeRS, since Se is a highly toxic element. Based on the lowest observed adverse effect level (LOAEL) of Se (913 μg/d), the dietary reference intake for Japanese in 2005 indicated 350 to 450 μg/d as the tolerable upper intake level (UL) of Se for adults. Since the energy content of the basal diet used in experiment 2 was estimated to be 3.86 kcal/g, 0.1 and 2.0 μg Se/g correspond to 0.026 and 0.52 μg Se/kcal respectively. Thus, based on the estimation that the energy intake of Japanese adults is 2,000 kcal/d, the intake of a diet with 0.1 or 2.0 μg Se/g in mice is considered to correspond to a human Se intake of 52 or 1,040 μg/d respectively. This indicates that a diet containing Se at a level of 0.1 μg/g causes a high Se intake, which exceeds not only the UL but also the LOAEL for Se. Accordingly, the inhibitory effect of Se on ACF formation at a level of 2.0 μg/g cannot be applicable to the human diet regardless of the Se source.

Since an additional Se intake of 52 μg/d probably causes no adverse effects on human health, the inhibitory effect of SeRS on ACF formation at 0.1 μg Se/g appears to be applicable to the human diet. However, the present results indicate only that supplementation with SeRS of a low Se diet at a level of 0.1 μg Se/g was effective at inhibiting the formation of DMH-induced ACF in the colon of mice. Moreover, supplementation with SeRS at 2.0 μg Se/g did not show a higher inhibitory effect than that at 0.1 μg Se/g. Since the average daily Se intake of the Japanese has been estimated to be about 100 μg/d, additional Se intake from SeRS may not be effective for cancer prevention.

Acknowledgment

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