

Selenium-enriched Japanese Radish Sprouts Influence Glutathione Peroxidase and Glutathione S-Transferase in an Organ-specific Manner in Rats

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Abstract: Selenium-enriched Japanese Radish Sprouts Influence Glutathione Peroxidase and Glutathione S-Transferase in an Organ-specific Manner in Rats: Hidetoshi HAMA, et al. Department of Preventive Medicine, Shinshu University School of Medicine—Selenium-enriched Japanese radish sprouts (Se-enriched JRS), in which Se-methylselenocysteine accounted for 80% of Se compounds, inhibited mammary tumorigenesis induced by 7,12-dimethylbenz[a]anthracene in rats. The effects of Se-enriched JRS on the oxidative stress-scavenging enzymes were investigated in rats. F344 female rats were fed test diets, in which Se-enriched JRS was added at 0, 2.4, 5.0, 8.8 or 12.5 ppm Se to commercial rodent chow for 3 wk. Glutathione peroxidase (GPx) and glutathione S-transferase (GST) in rat livers, kidneys and lungs were measured. Tissue Se concentrations at the highest Se dose (12.5 ppm) were high in order as follows: kidney > liver > lung. The diet at 12.5 ppm Se reduced the increase in body weight and, conversely, increased the liver weight. The Se test diets decreased hepatic and renal GPx activity at more than 2.4 ppm and 5.0 ppm, respectively. In contrast, the test diets increased pulmonary GPx activity at more than 2.4 ppm Se. The diets increased hepatic GST activity at more than 2.4 ppm Se dose dependently, whereas they reduced pulmonary GST activity at more than 2.4 ppm. The diet of 12.5 ppm Se induced GST Yp in all 3 organs and GST Yb1 in the liver. Thus, Se-enriched JRS influenced GPx and GST activity in a symmetrical manner in the livers and lungs of rats, with hepatic GST possibly affected, in part, by

the induction of GST Yb1.
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Key words: Glutathione S-transferase, Glutathione peroxidase, Kidney, Liver, Lung, Se-methylselenocysteine

An essential trace element, selenium (Se), has a potential cancer preventive effect^{1–3}. However, the fact that a continuous intake of Se compounds may lead to Se accumulation in the body, producing a cytotoxic effect, must also give considerable cause for concern^{4–6}. The major representative chemical forms of Se are selenite and selenate in inorganic forms, and selenomethionine, Se-methylselenocysteine (MeSeCys) and γ -glutamyl-MeSeCys in organic forms. The latter two, MeSeCys and γ -glutamyl-MeSeCys, are preferred to the others from the viewpoint of their potential cancer preventive effects and safety, because of their lower accumulation level in tissues as well as their wide intervals between cancer inhibiting doses and toxic ones^{7–9}.

Many vegetables are known to have cancer preventive effects. Se-enriched garlic has breast cancer preventive potential^{10–12}, while Se-enriched broccoli florets have colorectal and breast cancer preventive potential^{13–15}; their major chemical form of Se is MeSeCys^{16–18}. These plants can convert inorganic Se such as selenite or selenate to the organic forms, thus enhancing their anti-cancer power.

Hitherto, although many vegetable varieties have been cultivated to investigate the preventive efficacy of the Se-enriched vegetables against cancer, their cultivation in soil has proved unsuccessful because of the inefficiency of Se absorption from the soil^{16, 19}. As an alternative method, hydroponic culture has been developed²⁰. Using the hydroponic methods, we also developed a useful Se-added fertilizer (Japan Patent No. 2969128) through the

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cultivation of Japanese radish sprouts (JRS). To our surprise, Se absorption proved to be considerably high, with the major chemical form in the vegetable being MeSeCys at a level of almost 80% in the sprouts²¹. Moreover, the Se-enriched JRS significantly inhibited 7,12-dimethylbenz[a]anthracene-induced mammary carcinogenesis in rats²¹. However, the mechanisms underlying the cancer preventive effect of Se-enriched JRS are not fully understood.

Se itself is known as a metabolic inactivator of peroxides and carcinogens, an inducer of cell death, an anti-angiogenic agent, a promoter of an anti-tumor substance, and an activator of the cellular immunity². Regarding the mechanism of metabolic inactivation, there are two candidate enzymes. One is glutathione peroxidase (GPx), a kind of selenoenzyme that includes selenocysteine, and its activity is a good index of Se bioavailability. Any ability that can remove hydrogen peroxides and lipid peroxides contributes to cancer inhibition, since peroxidative damage is associated with cancer^{4, 22, 23}. Indeed, the enhancement of GPx activities in livers and kidneys by Se-enriched vegetables has been reported^{13, 24, 25}. In contrast, there are some contradictory findings that conclude that GPx activity may be an inappropriate index of the cancer inhibitory effect because the optimum dosages of Se compounds to inhibit cancer are greater than the corresponding optimum dosages of Se compounds for influencing GPx activities^{1, 2, 26-28}. The other candidate enzyme is glutathione S-transferase (GST) that can remove superoxides and the carcinogenic intermediates of xenobiotics, and it is thought to be useful for cancer inhibition^{29, 30}. Since the ingestion of Se-enriched garlic bulbs enhanced GST activities in livers and kidneys²⁹, it is of interest to determine whether Se-enriched JRS induces these enzyme activities. In addition, there is no information concerning the effects of Se-enriched sprouts on GST isozymes such as GST Ya, GST Yc, GST Yk, GST Yb and GST Yp.

In this study, we clarified the effects of the Se-enriched JRS we cultivated on two antidotal enzymes, GPx and GST, in livers, kidneys and lungs of rats, and explained how the ingestion of these sprouts affected these enzymes in an organ-specific manner.

Materials and Methods

Production of Se-enriched JRS and test diets

Japanese radish sprouts (*Raphanus sativus*) were seeded in flat containers 3 inches deep together with growth fertilizer. After sprouting, the fertilizer was changed to Se-added fertilizer (Japan Patent No. 2969128). When the sprouts had grown to 10 cm in about 7 d, they were harvested, immediately frozen and lyophilized. The chemical form of Se was mainly MeSeCys (80%)²¹, and the Se-concentrations analyzed by microwave induced plasma mass spectrometry (MIP-MS) were 330 ppm Se of

dry weight. Four kinds of test diets, all of them Se-enriched JRS-added diets, were prepared by adding the lyophilized JRS to commercial rodent F2 chow (basal diet) (Funabashi Farm Co., Ltd., Chiba, Japan) adjusted according to the National Research Council Guidelines: JRS-added diets (0, 2.4, 5.0, 8.8 and 12.5 ppm Se) were formulated by adding to the basal diet, in which the Se concentration measured by MIP-MS was 0.67 ppm. Therefore, the final Se concentration in each test diet must be roughly calculated by including the level of the basal diet. The rats fed the basal diet alone (0 ppm Se-added diet) were designated as the control group.

Animals

This study was conducted according to the Guidelines for Animal Experiments of the Shinshu University Animal Center. F344 virgin female rats at 7 wk of age (n=21) were purchased from Japan SLC, Inc. (Shizuoka, Japan). All rats were housed individually in stainless steel wire-bottomed cages in a room with controlled temperature, light and humidity. Then, at 8 wk of age, they were divided into five groups and fed either the basal or one of the test diets for 3 wk. All rats were sacrificed by decapitation, and the livers, kidneys and lungs were quickly removed. These organs were kept at -80°C until use.

Measurement of Se concentration in organs

Se concentrations in the livers, kidneys and lungs were measured by MIP-MS according to the method of Chiba and Shinohara³¹.

Preparation of sub-cellular fractions and assay of enzyme activities

Tissues were homogenized in three volumes (w/v) of 0.25 M sucrose-10 mM phosphate buffer (pH 7.4) with an ultrasonic cell disrupter (Yamato Scientific Co., Ltd., Tokyo, Japan). Fractions of enzyme source were prepared at 4°C by differential ultracentrifugation. Supernatants of the first centrifugation of homogenates at 10,000 × g for 10 min (10,000 × g supernatant fractions) were further centrifuged at 105,000 × g for 1 h to obtain the cytosolic fractions. The 10,000 × g supernatants and cytosols were used for the measurement of GPx and GST activities, respectively. Protein concentrations in each fraction were measured by the Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA, USA) with bovine serum albumin (BSA) as a standard. GPx activities were measured using the BIOXYTECH® GPx-340™ (OXIS International, Inc., Foster City, CA, USA), and GST activities were measured using 1-chloro-2,4-dinitrobenzene as a substrate according to the method of Habig and Jakoby³².

Western blot analysis

Visceral cytosol fractions adjusted to 10 µg protein

Table 1. Body and organ weights of rats fed selenium-enriched Japanese radish sprouts (g)

Se levels added to diet	0 ppm (Basal diet) n=4	2.4 ppm n=4	5.0 ppm n=4	8.8 ppm n=4	12.5 ppm n=5
Body	165.5 ± 8.6	166.5 ± 16.9	154.0 ± 6.7	151.3 ± 9.4	149.4 ± 6.1*
Liver	6.07 ± 0.29	6.16 ± 0.81	6.28 ± 0.40	6.60 ± 0.61	6.81 ± 0.52*
Kidney	1.21 ± 0.09	1.16 ± 0.10	1.10 ± 0.04	1.05 ± 0.08*	1.05 ± 0.03*
Lung	0.71 ± 0.07	0.73 ± 0.07	0.71 ± 0.05	0.72 ± 0.04	0.73 ± 0.12

Each value represents mean ± SD. * Significantly different from control, $p < 0.05$.

were subjected to 12.5% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. The primary polyclonal antibodies to GST A1 (GST Ya), GST A3 (GST Yc), GST A4 (GST Yk), GST M1 (GST Yb1) and GST P1 (GST Yp) were purchased from Biotrin International, Co., Ltd., Dublin, Ireland. After blocking with 3% skim milk, membranes were incubated with the primary antibodies (1:1,000), respectively, followed by incubation with alkaline phosphatase-conjugated goat anti-rabbit IgG antibody (1:5,000; Jackson Immuno Research, West Grove, PA, USA; product number 111-055-003). 1-Step™ NBT/BCIP (Pierce Biotechnology Inc., Rockford, IL, USA) was used for the substrate of alkaline phosphatase. Each band was quantified using a densitometer, [Lane & Spot Analyzer, version 5.0 (ATTO Corporation, Tokyo, Japan)].

Statistics

All data are presented as means ± standard deviation (SD) in Tables and means +SD in Figs. 1 and 2. One-way analysis of variance was performed, followed by Dunnett's analysis, to compare the results of the control group with those of the Se-enriched groups. Probability values of < 0.05 were considered to be significant.

Results

Body and organ weights

Table 1 shows the body and organ weights after 3 wk of feeding with the basal (control group) or test diets including different concentrations of Se, i.e., Se-enriched JRS. At the beginning of the test diet feeding, no significant differences were observed in body weights at the baseline. However, after 3 weeks of feeding, body weights in the highest Se feeding group (12.5 ppm Se) were significantly lower than those in the controls, suggesting that the diet with the highest Se level inhibited the growth of rats. Test diets with added Se at levels of 5.0 and 8.8 ppm seemed to retard increase in the body weights, but not to a statistically significant degree. The lowest Se-added diet (2.4 ppm Se) did not influence the increase in body weights.

The liver weights of rats fed 12.5 ppm Se-added diet were significantly greater than those of controls, and the ratio of liver per body weight increased after feeding with the highest Se diet, suggesting that the highest Se diet increased liver weight. However, macroscopically, there were no changes in the liver, so we considered that Se-enriched JRS did not cause liver damage. In contrast, kidney weights of rats fed 8.8 and 12.5 ppm Se-added diets were significantly lower than those of controls. Since there was no significant difference in the ratio of kidney per body weight, the lower kidney weights were accompanied by decrease in body weights. No Se-enriched diet influenced the lung weights of rats.

Se concentrations in organs

Se concentrations in livers, kidneys and lungs of rats fed basal and the highest Se (12.5 ppm) containing diets are shown in Table 2. The highest Se concentration was seen in the kidneys, and the lowest in the lungs.

GPx activities

When GPx activities were compared among liver, kidney and lungs of the control group, they were found to be greatest in the livers, and those in kidneys and lungs were one fifth and under one tenth those of the livers, respectively (Fig. 1). Se-enriched diets dose-dependently decreased GPx activities in the livers and kidneys, though the effect on kidneys was not in a clear dose-dependent manner. In contrast, the Se diets increased activities in the lung, but not dose-dependently.

GST activities

In organs of the control group, GST activities were slightly higher in the livers than in the kidneys and lungs, but no difference was observed in the activities between the latter two organs (Fig. 2). Se-enriched diets with more than 2.4 ppm Se, dose-dependently increased GST activities in the liver of rats, while the same dosages decreased GST activities in the lungs. Test diets with more than 5.0 ppm Se seemed to increase GST activities in the kidneys, but not in a statistically significant manner.

Table 2. Se concentrations in the livers, kidneys and lungs of rats fed selenium-enriched Japanese radish sprouts (ppm)

Se levels added to diet	0 pm (Basal diet) n=5	12.5 ppm n=5
Liver	1.36 ± 0.07	4.37 ± 0.39*
Kidney	1.41 ± 0.05	6.55 ± 0.64*
Lung	0.45 ± 0.02	2.05 ± 0.24*

Each value represents mean ± SD.

* Significantly different from control, $p < 0.05$.

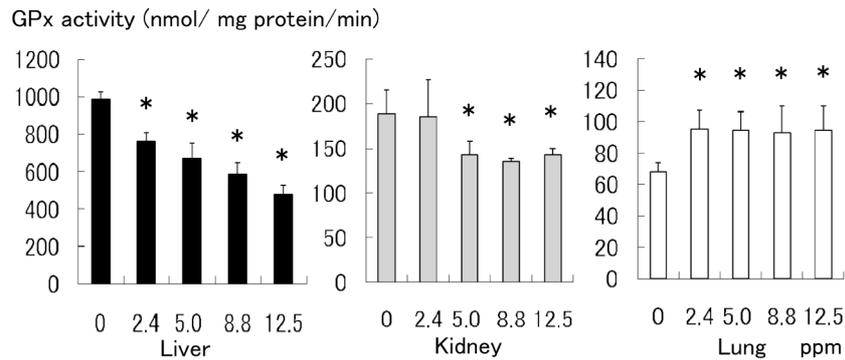


Fig. 1. Glutathione peroxidase (GPx) activities in livers, kidneys and lungs of rats after being fed diets with selenium-enriched Japanese radish sprouts (Se-enriched JRS). Abscissa shows Se levels added to each diet. Numbers in each group were 4 except for the 12.5 ppm Se group in which there were 5. Columns and bars represent means +SD. *Significantly different from basal diet group (0 ppm Se-added diet) ($p < 0.05$).

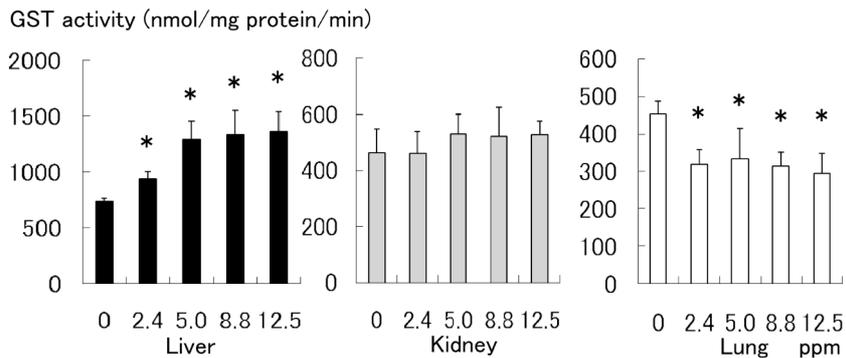


Fig. 2. Glutathione S-transferase (GST) activities in livers, kidneys and lungs of rats after being fed diets with selenium-enriched Japanese radish sprouts (Se-enriched JRS). Abscissa shows Se levels added to each diet. Numbers in each group were 4 except for the 12.5 ppm Se group in which there were 5. Columns and bars represent means +SD. *Significantly different from basal diet group (0 ppm Se-added diet) ($p < 0.05$).

Table 3. Comparative expression of glutathione S-transferase subunits in rats fed selenium-enriched Japanese radish sprouts

Se levels added to diet	0 ppm (Basal diet) ^a n=5	12.5 ppm n=5	
Liver	Ya	1.00 ± 0.07	1.28 ± 0.27
	Yc	1.00 ± 0.16	1.31 ± 0.29
	Yk	1.00 ± 0.17	1.19 ± 0.02
	Yb1	1.00 ± 0.39	3.31 ± 0.60*
	Yp	Not detected	↑
Kidney	Ya	1.00 ± 0.13	1.10 ± 0.17
	Yc	1.00 ± 0.37	1.39 ± 0.14
	Yk	1.00 ± 0.48	1.23 ± 0.08
	Yb1	Not detected	Not detected
	Yp	1.00 ± 0.12	2.14 ± 0.13*
Lung	Ya	Not detected	Not detected
	Yc	1.00 ± 0.44	1.05 ± 0.58
	Yk	1.00 ± 0.19	1.31 ± 0.22
	Yb1	Not detected	Not detected
	Yp	1.00 ± 0.30	1.96 ± 0.22*

^a Intensity of bands obtained from rats fed basal diet was designated as 1.0 (mean ± SD). ↑, increased (ratio unable to be calculated). *Significantly different from control, $p < 0.05$.

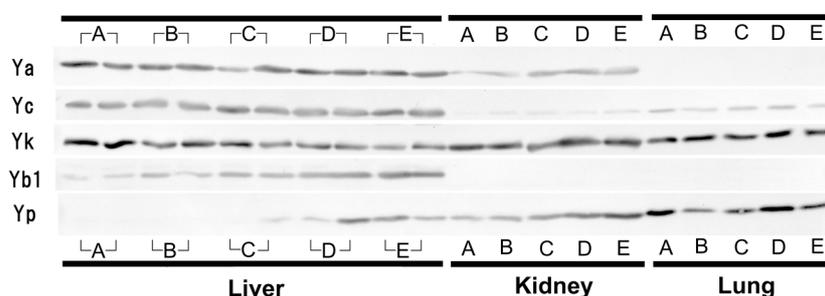


Fig. 3. Immunodetection of glutathione S-transferase subunits with polyclonal antibodies to rat GST Ya, GST Yc, GST Yk, GST Yb1 and GST Yp. Se dosages are as follows: (A), 0 ppm; (B), 2.4 ppm; (C), 5.0 ppm; (D), 8.8 ppm; (E), 12.5 ppm.

GST isozymes

There was no band stained by anti-GST Yp in the livers of control rats or in those fed diets with 2.4 ppm Se (Table 3 and Fig. 3). However, the band clearly appeared in liver samples of rats fed the test diets containing more than 5.0 ppm Se. In kidneys and lungs, GST Yp protein was constitutively expressed, and was enhanced by test diets to which more than 8.8 ppm Se were added.

In contrast to GST Yp, bands of anti-GST Yb1 were constitutively expressed in the livers of control rats, and enhanced by feeding of Se-added test diets with more

than 5.0 ppm Se. Similar to the expression of GST Yp in livers, a band stained by anti-GST Yb1 was not detected in the kidneys or lungs of control rats, and feeding of Se-enriched diets had no effect on this level.

In livers and kidneys of the control group, bands for GST Ya, GST Yc and GST Yk were observed, while in lungs, only bands for GST Yc and GST Yk were detected, suggesting that these three proteins were constitutively expressed in livers and kidneys, while the latter two were constitutively expressed in lungs. However, expressions of GST Yc in kidneys and lungs were remarkably lower

than those in the liver, and Se-added diets did not affect the expression of these isozymes.

Discussion

This study clearly shows the following: Se-enriched JRS influenced GPx and GST activities in the livers, kidneys and lungs of rats in an organ-specific manner. The influence of the diets on livers were completely opposite to those on lungs: in livers, GPx activities were reduced, while GST activities were increased by raising the Se dosages in the diets, whereas in lungs, the former were increased and the latter were reduced. However, in kidneys, GPx activities were only slightly reduced, while GST activity was retained even at the highest intake of Se. In a preliminary study, we verified that JRS itself influenced neither body and organ weights nor GST and GPx activities (data not shown), suggesting that the effects on GST or GPx observed in this study are due to MeSeCys in Se-enriched JRS.

In contrast to the increased, retained or reduced GST activities in the livers, kidneys and lungs, respectively, after feeding of Se-enriched diets, the expression of GST Yp followed a similar course in all the organs we investigated: in livers, expression of GST Yp was induced by diets with more than 5.0 ppm Se, while in kidneys and lungs GST Yp expression was slightly enhanced. GST Yb1 expression was enhanced by diets with more than 5.0 ppm Se in livers only. The expressions of the other isozymes investigated were not changed in any organ by the Se-added diets. Taken together, enhancement of hepatic GST Yb1 may reflect enhancement of hepatic GST activities for 1-chloro-2,4-dinitrobenzene, and the involvement of hepatic GST Yp may be very small. This deduction may also be supported by the finding that the constitutive activity of GST for 1-chloro-2,4-dinitrobenzene in livers was greater than that in kidneys or lungs, where GST Yb1 was not expressed constitutively, while GST Yp was. However, our findings could shed no light on the reason why GST activities were reduced in lungs following Se-enriched JRS administration. Since the contributions of the other GST isozymes such as GST Yb2 to the activities in lungs were not investigated, further study may be needed to clarify the reason.

In regard to the relationship between Se concentration in the tissues and GST activities, 1 ppm Se-enriched broccoli did not influence either hepatic or renal GST activities¹³, whereas those activities were increased dose-dependently in the livers and kidneys of rats until the Se attained tissue levels of 7.3 ppm and 8.7 ppm, respectively, corresponding to a diet of 3 ppm of Se-enriched garlic²⁹. That finding for the liver was very similar to the current one, in which a Se concentration in the liver of 4.37 ppm increased hepatic GST activities. However, in the kidneys, no significant increase in GST

activities was observed, a finding that differs considerably from past reports²⁹. Since we could not resolve this discrepancy, further study may be required to determine whether the difference arises from differences in cultivation methods.

Se-enriched JRS significantly induced the expression of GST Yp in all the organs investigated. It is possible that GST Yp would be helpful in the detoxification of carcinogens because of its ability to catalyze conjugation reactions for carcinogens or peroxides³³⁻³⁵. Se-enriched JRS inhibited 80% of rat mammary carcinogenesis induced by 7,12-dimethylbenz[a]anthracene at a Se dose of 8.8 ppm in the diet²¹. Since hepatic GST Yp was induced after feeding of test diets with more than 5.0 ppm Se, the GST Yp induced might be involved in the hepatic detoxication of 7,12-dimethylbenz[a]anthracene.

On the other hand, the induction of GST Yp might be an outcome of Se toxicity. The maximum tolerable dose of a selenocompound is defined as one that can cause a body weight loss of 7–10% over a 4-wk administration⁷. Since our observation period of the test diets including MeSeCys lasted only 3 wk, it was of insufficient duration to reliably judge toxicity. However, the 12.5 ppm Se-added diet reduced body weight by 9.7%, and the 8.8 ppm diet reduced it by 8.6%, which clearly fall within the definition of toxic dosages. Although the 5.0 ppm Se-added diet reduced body weight by 6.7%, this reduction may also indicate a toxic dose because of the exposure to Se in the diet was 1 wk shorter than that required for the toxicity assessment. Thus, Se-enriched JRS of more than 5 ppm Se are by definition toxic, and GST Yp began to increase in livers at these concentrations, and the maximum tolerable dose of MeSeCys in rats is thought to be 5.0 ppm⁷.

In contrast to GST activities, the effects of Se-enriched JRS on GPx activity were more complicated. The reason why Se-enriched JRS decreased GPx activity in livers and kidneys in the current study is unknown. Administration of diet including Se-enriched broccoli at a Se concentration of 0.1–1.0 ppm enhanced hepatic GPx activity^{13, 14}. Recently, Yoshida et al. reported that administration of diet including Se-enriched JRS at a Se concentration of 0.15 ppm increased hepatic GPx activity³⁶. In the kidneys, Se-enriched broccoli in diet at Se levels of 1 ppm also increased those activities¹³. These findings suggest that GPx activity may be enhanced at relatively low Se concentrations in the diet, 1.0 ppm or less, and that the activity may be reduced following administration of a higher Se concentration in the diet, for example, 2.4 ppm Se. In the experiments previously reported, Se concentrations of diets administered to control rats were set extremely low^{13, 14, 29, 36}, and might have been insufficient for enhancing GPx activity, while the concentration in our study was 0.67 ppm in the basal diet, which might have been sufficient for enhancing GPx

activity. In regard to the relationship between tissue Se concentration and GPx activity, hepatic GPx activity increased at the hepatic Se concentration of 2.5 ppm compared to that at 0.2 ppm levels¹⁴. Thus, GPx activities might also increase in lungs because lung tissue Se concentrations were relatively lower than those in livers or kidneys, in which such activities either decreased or remained at the control levels.

Several problems are apparent with the current study: the histopathological evaluation of all three organs was not performed; the relationship between tissue Se concentration and GST or GPx activity should be investigated; and the effects of Se compounds other than MeSeCys need to be investigated.

In conclusion, similar to Se-enriched garlic and broccoli, and Se-enriched JRS cultivated by our group enhanced GST activities in the livers of rats, which may prove to be of significance in the chemoprevention of breast cancer induced by 7,12-dimethylbenz[a]anthracene in rats. However, the finding of the enhancement of GST activities was not always the same in the other organs. From the viewpoint of the effects on body weight as well as on the induction of GST Yp isozyme, feeding diets containing less than 5 ppm Se (perhaps reducing it to around 2.4 ppm), may be recommended for future experimental investigations of cancer prevention in rats.

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