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Anticarcinogenic Activity of Selenium-Enriched Green Tea Extracts in Vivo

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Both selenium and green tea have been shown to have potential antitumor effects. Here we have investigated the anticarcinogenic effect of the selenium-enriched green tea extract (Se-TE) in a Kunming mice model transplanted with human hepatoma cells HepG2. Mice were assigned to 8 groups consisting of 10 mice each after tumor cell inoculation. The control group received only water, whereas the remaining groups received regular green tea extract (RT), Se-TE which was produced by fertilization with selenite on tea leaves, selenite, and RT + selenite. After the mice were fed intragastrically with these agents for 8 days, tumor growth in RT-, Se-TE-, and selenite-fed mice was significantly suppressed, compared with that in control mice ($P < 0.001$). Supplementation with Se-TEs and selenite was able to elevate mice blood and liver Se concentrations, but did not significantly enhance selenoprotein glutathione peroxidase and other antioxidant enzyme superoxide dismutase activity in mice blood and liver. These results suggest that the antitumor function of Se-TEs may be attributed to the oxidative stress induced by selenium and green tea components in a suitable selenium supplementation pathway.

KEYWORDS: Se-enriched tea; hepatocellular carcinoma; chemoprevention; glutathione peroxidase

INTRODUCTION

Selenium, an essential trace element for animals and humans, has received considerable attention for its potential role as a chemopreventative agent (*1*). Epidemiological studies have revealed an inverse association between dietary selenium intake and cancer risk in humans (*1*−*4*). Animal studies also showed that selenium deficiency in the diet can cause a decrease in weight in pregnant rats (*5*), and growth retardation induced by selenium deficiency is associated with impaired bone metabolism and osteopenia (*6*). An adequate Se intake is associated with a reduction in the risk of cancer (*7, 8*), and selenium and its compounds are also reported to have a protective effect in the initiation phase of colon cancer and are able to inhibit tumorigenesis in some tissues of animals (*9*−*11*). For this reason, Se fertilization has been employed to raise the Se content in plant food and drinks in certain parts of the world in which Se is deficient in the soil. Selenium was first reported to be enriched in garlic by Ip and co-workers, and Se-enriched garlic was able to exert mammary cancer prevention and inhibit the early stage of mammary carcinogenesis (*12*, *13*). This research was followed by Se fertilization into broccoli which could decrease intestinal tumorigenesis in multiple intestinal neoplasia mice (*14*). Since then, selenium enrichment has successfully been employed on other foodstuff including Se-enriched yeast, mushroom species of the genus *Ganoderma*, and mycelia of *Pleurotus ostreatus* (*15*−*17*).

Tea is a widely consumed beverage and has been shown to possess antipyretic, diuretic, and several other pharmacological activities. The major constituents of green tea are polyphenols which are responsible for higher antioxidant and antimutagenic properties (*18*). Wang and co-workers reported that green tea GTP has substantial anti-skin-tumor-initiating activity against PAHs (*19*) and could prove useful in protecting against some forms of human cancer (*20*).

Since both selenium and green tea have been reported to exhibit antigenotoxic and cancer chemopreventive properties, it is very interesting to know whether a combination of selenium and green tea can obtain a better protection-enhancing effect than selenium or tea alone. Such efforts were first put into practice by Hu's group. Green tea fertilized by selenium contained much more selenium and some other bioactive contents including vitamin C and polyphenol (*21*). In vitro antioxidant assay also demonstrated that Se-enriched green tea extracts showed a higher protection effect against lipid peroxidation and higher radical-scavenging activities (*22*). Meanwhile, *Salmonella* assay on Se-enriched green tea extracts indicated dose-dependent inhibition of IQ-induced mutagenesis in the presence of rat liver S9 and was significantly more effective than regular green tea tested under the same conditions. An enhancing effect of selenium in combination with green tea in

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vitro was also found in this experiment (*23*). However, little is reported on the anticarcinogenic effect of Se-enriched tea and its related mechanisms in animals.

Therefore, the present study was designed to compare the effect of green tea extracts with and without Se application on the growth of HepG-2-transplanted mice. The aim of this study was to see whether Se-enriched tea can obtain an enhancing anticarcinogenic effect, and by measurements of Se bioavailabilities and antioxidant systems including glutathione peroxidase and superoxide dismutase, we are hoping to present some mechanisms for understanding the anticarcinogenicity of Se-enriched tea and to generate data that may lead to future investigations on the production of Se-enriched tea as a nutritional adjuvant in cancer prevention.

MATERIALS AND METHODS

Chemicals. Sodium selenite and sodium selenate were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals used were of analytical grade.

Preparation of Se-Enriched Green Tea Extract (Se-TE). The preparation of Se-TE has been described (*24*). Briefly, selenium fertilizer prepared from selenite was sprayed on the old tea leaves. After 10 days of fertilization, the newly growing bud and young leaf were harvested and processed into commercial roasted green tea. Water was used as the control to produce regular tea (RT). A 1 kg portion of green tea was ground into a fine powder and then extracted with 8 volumes of boiling water three times. The tea extracts were combined and concentrated in vacuo till the final volume was 2 L. The concentrations of selenium and total water-soluble solids in the tea concentrates were determined according to a method described previously (*22*), and then, the concentrated tea extracts were assigned for administration to mice.

Animals and Cell Culture. Kunming mice (Swiss albino mice origin), 4−6 weeks old, initially weighing 18−20 g, were purchased from the Nanjing Qinglongshan Animal Feed Centre (Nanjing, China) and housed individually in a room with a controlled temperature (22 °C) and a 12 h/12 h light/dark cycle. The mice were fed ad libitum a basal diet described by Spolar (*25*) with negligible Se. The hepatoma cell line (HepG-2) was kindly supplied by the Shanghai Institute of Materia Medica, Chinese Academy of Sciences. The cells were routinely maintained in RPMI-1640 containing 10% fetal bovine serum, 100 U·mL^{-1} penicillin, and 100 μg·mL^{-1} streptomycin at 37 °C in a 5% CO_2 incubator.

Animal Experiment. Xenografts were initiated by subcutaneous injection of 2×10^6 hepatoma cells (HepG-2) into the right flanks of four healthy mice. After 1 week, the tumors were aseptically dissected and mechanically minced to obtain a monolayer cell suspension of 1.0×10^8. A 200 μL portion of this HepG-2 cell was subcutaneously implanted on the right upper limb of each mouse after 2 days of acclimation. All animals were then randomly divided into 8 groups (10 mice per group). The mice received different treatments daily by intragastric administration (0.6 mL) for 8 days: group 1, saline; group 2, RT extracts; groups 3−5, Se-TE with Se doses of 0.167 (L), 0.333 (M), and 0.669 (H) μg of Se/mL; group 6, RT plus selenite with a final Se concentration of 0.333 μg/mL; group 7, selenite solution (0.333 μg of Se/mL); group 8, 5-fluorouracil (5-Fu). The final administered concentrations of selenium and water-soluble solids in the diet by groups 2−7 mice are listed in **Table 1**. Body weights and food intake were recorded daily during the experiment. On the ninth day after 24 h of deprival of drug administration, blood was collected by venous puncture in mice under diethyl ether anesthesia, and all the animals were killed by cervical dislocation. The tumor, spleen, and thymus were removed and weighed for evaluation of antitumor activity and immunocompetent organ functions. The liver was excised, frozen in liquid nitrogen, and stored at −80 °C for further biochemical assay. The inhibition rate (IR) of the tumor was calculated by comparing the tumor masses of the experiment and control groups: IR (%) = (1 − mean tumor mass in the experiment groups/mean tumor mass in the negative control) × 100%. The effect of Se-TE on the mouse immunological activities were also evaluated by the ratios spleen weight (mg)/body weight (g) and thymus weight (mg)/body weight (g).

Table 1. Se-TE Contents Applying to Tumor-Bearing Mice[a]

tea	total tea water extract (mg/mL)	selenium content (μg/mL)
RT	36.00 ± 2.43	0.005 ± 0.001
Se-TE (L)	39.40 ± 3.17	0.167 ± 0.002 a
Se-TE (M)	78.81 ± 4.56 ab	0.335 ± 0.002 ab
Se-TE (H)	157.6 ± 7.32 abc	0.669 ± 0.013 abc
selenite solution	0	0.333 ± 0.002 abd
RT + selenite	36.43 ± 1.75 d	0.330 ± 0.017 abd

[a] Values are the mean ± SD. Letters in a column indicate the significant difference: a, different from RT, $P < 0.01$; b, different from Se-TE (L), $P < 0.01$; c, different from Se-TE (M), $P < 0.01$; d, different from Se-TE (H), $P < 0.01$.

Table 2. Effect of Se-TE Administration on the Body Weight (BW) and Organ Weight/Body Weight Ratio of the Spleen and Thymus Following 8 Days of Feeding[a]

treatment	food intake (g)	initial BW (g)	terminal BW (g)	spleen/BW	thymus/BW
control	2.48 ± 0.28	20.0 ± 1.59	19.3 ± 1.32	5.41 ± 1.32	2.58 ± 1.05
RT	3.16 ± 0.73	19.8 ± 1.61	18.4 ± 1.57	6.29 ± 2.70	1.70 ± 0.44
Se-TE (L)	3.08 ± 0.21	19.9 ± 1.39	20.4 ± 1.95	4.53 ± 1.72	1.83 ± 0.58
Se-TE (M)	2.93 ± 0.33	20.0 ± 1.45	17.10 ± 1.67	4.64 ± 2.03	1.78 ± 0.46
Se-TE (H)	2.56 ± 0.45	20.0 ± 2.01	18.1 ± 1.43	3.80 ± 0.84	1.64 ± 0.64
RT + selenite	2.98 ± 0.27	20.0 ± 1.71	18.2 ± 1.67	4.75 ± 0.81	1.69 ± 0.48
selenite	2.78 ± 0.42	20.2 ± 1.69	20.4 ± 1.70	7.19 ± 2.23 a	2.52 ± 1.00
5-Fu	2.80 ± 0.39	20.2 ± 1.86	19.2 ± 1.45	5.00 ± 1.03	1.70 ± 0.67

[a] Values are the mean ± SD. Letters in a column indicate significant difference: a, different from Se-TE (H), $P < 0.01$.

Biochemical Assay. Blood and liver selenium concentrations were determined by the method of atomic absorption spectrophotometry (*26*). A mixed acid digestion procedure was used to decompose organic material. Sodium borohydride, a redesigned hydride generator, and an electrically heated absorption tube were used for H_2Se evolution and conversion to atomic selenium, which was finally recorded by atomic absorption spectrophotometry.

Superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities in the blood and liver cytosolic fraction were assayed by using commercially available colorimetric kits according to the manufacturer's protocol (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Determination of Hematological Parameters. The effect of Se-enriched tea on hematological parameters was also studied in the mice of all the groups. Hematological analysis was performed using an automatic hematological analyzer (Cell-Dyne 3500, Abbott). Blood was collected from three mice of all the groups by puncturing the retro-orbital plexus. Hematological parameters including the hemoglobin content and total count of red blood cells (RBCs) and white blood cells (WBCs) were measured.

Statistical Analysis. Data are expressed as the mean ± SD. All analyses were performed using the statistical package SPSS 13.0.1 (SPSS, Chicago, IL). The evaluation of the data was done with one-way analysis of variance (ANOVA) followed by LSD and the Bonferroni post hoc test for multiple comparisons. Differences are considered significant at the level of $P < 0.05$.

RESULTS

To investigate the effect of Se-TEs on tumor growth in vivo, the mice were administrated the aqueous extracts of Se-TEs produced from selenite fertilizer. The final concentrations of components of regular green tea and Se-TE for administration are listed in **Table 1**. The daily administrated selenium concentrations were 5.0, 10.0, and 20.1 (μg of Se/kg of body weight)/day. Following administration, we found no significant difference in body weight among the groups throughout the

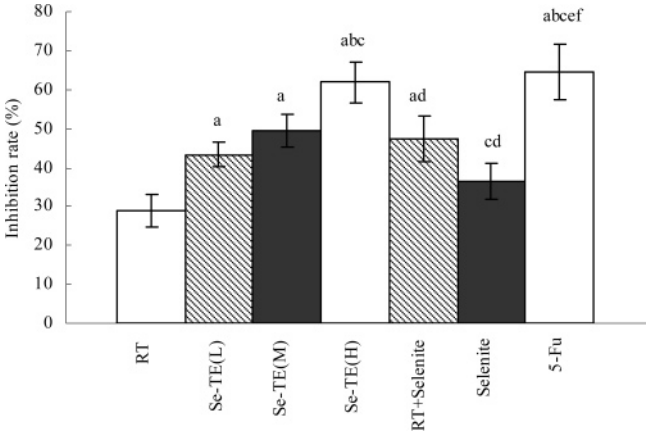

Figure 1. Anticarcinogenic activities of Se-enriched green tea against mice with transplanted hepatocarcinoma tumor in terms of inhibition rate (%). Values are the mean ± SD of nine independent determinations. Letters indicate significant difference: a, different from RT, $P < 0.01$; b, different from Se-TE (L), $P < 0.01$; c, different from Se-TE (M), $P < 0.01$; d, different from Se-TE (H), $P < 0.01$; e, different from Se-TE (Se + selenite), $P < 0.01$; f, different from selenite, $P < 0.01$.

experiment (**Table 2**). At necropsy, there also were no big alternations in the liver, spleen, and thymus weights among the groups. These data suggested that supplementation with regular tea or Se-enriched tea did not affect the growth of the mice.

Following implantation with HepG-2, there was noticeable growth of tumors due to proliferation of tumor cells. As a result, the tumor weight in mice administrated water was found to be the highest (1.84 ± 0.20 g) (data not shown). Comparison with the control showed that all treatments led to pronounced reduction of the tumor weights (**Figure 1**) ($P < 0.05$). The tumor inhibition rate decreased in the order 5-Fu > Se-TE (H) > Se-TE (M) > RT + selenite > Se-TE (L) > selenite > RT. Se administration caused significantly higher inhibition of tumor growth than RT and selenite alone. This inhibitory effect increased significantly with increasing dose of Se-TE administration ($R = 0.999$, $P < 0.001$). However, the rate for mice fed the same dose of Se-TE (M), 10 μg/kg of body weight, did not differ from that for mice fed RT + selenite, while there was a significant difference present between the rates for mice fed selenite and Se-TE or RT + selenite ($P < 0.05$). This result indicated that the inhibition effect provided by Se-TEs may be due to an addictive effect of green tea components with selenium (**Figure 1**). In this experiment, 5-Fu adopted as a positive control provided the highest tumor inhibition of 64.61 ± 1.35% compared to that of Se-TE (H) of 61.8 ± 1.73.

Bioavailabilities of selenium from RT, Se-TE, RT + selenite, and selenite were also measured in terms of Se concentration in the blood and liver (**Figure 2**). Comparing the control and regular tea, sodium selenite treatment resulted in a dramatic increase of Se in the blood and liver. Interestingly, the Se bioavailability is also dependent on the form of Se and the Se dose. Se in the blood and liver of the mice increased dramatically after the treatment with Se-TE and selenite, while no difference was found among Se-TE, RT + selenite, and selenite. Mice fed with water and 5-Fu had significantly lower Se concentrations in both the blood and liver.

The biological functions of tea and selenium are normally attributed to their antioxidant activity; thus, we measured antioxidant enzyme SOD and Se-containing enzyme GPx activities. As shown in **Figure 3**, GPx activity in the blood and liver of mice receiving treatments had slight increases compared to that of the negative control. However, compared with

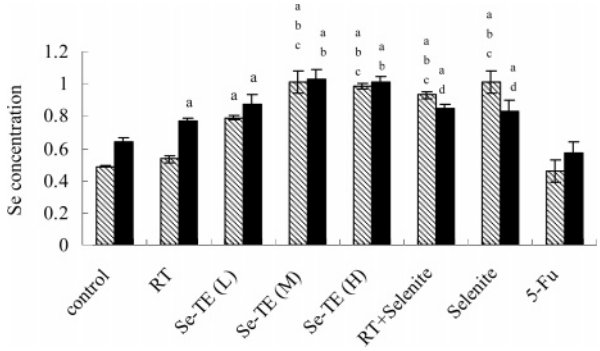

Figure 2. Effect of Se-TE on selenium concentrations in mouse blood and liver: (gray bars) blood Se concentration (μg mL^{-1}); (black bars) liver Se concentration (μg g^{-1}). Values are the mean ± SD of three independent determinations. Letters indicate significant difference: a, different from the corresponding control group, $P < 0.01$; b, different from the corresponding RT, $P < 0.01$; c, different from the corresponding Se-TE (L), $P < 0.01$; d, different from the corresponding Se-TE (M), $P < 0.01$.

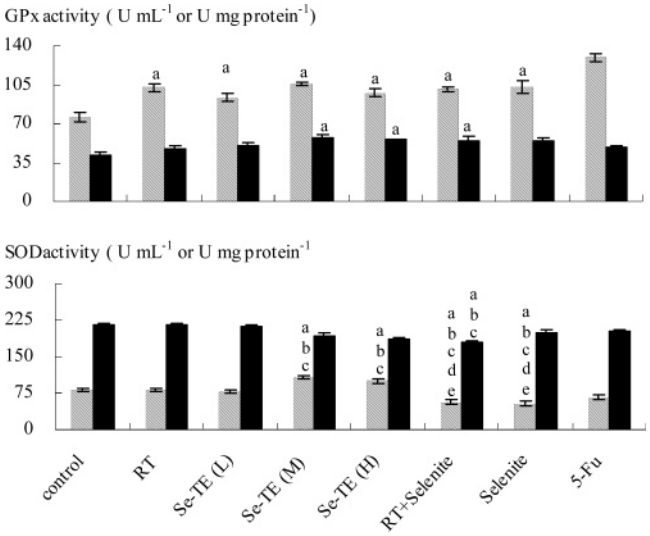

Figure 3. Effect of Se-TE on GPx and SOD activities in mouse blood (hatched bars) and liver (black bars). Values are the mean ± SD of three to nine independent determinations. Letters indicate significant difference: a, different from the corresponding control group, $P < 0.01$; b, different from the corresponding RT, $P < 0.01$; c, different from the corresponding Se-TE (L), $P < 0.01$; d, different from the corresponding Se-TE (M), $P < 0.01$; e, different from the corresponding Se-TE (H), $P < 0.01$.

113.07 ± 5.12 U mL^{-1} in healthy mouse blood, the GPx activities were reduced for all groups except the 5-Fu group. This decrease also happened in the liver of all groups with a GPx activity of 74.09 ± 3.01 U mg^{-1}. There is no big difference in blood GPx activity in mice receiving regular tea, Se-TE, and selenite (**Figure 3**, top). Regular tea intake did not affect the SOD activity in the mouse blood and liver (**Figure 3**, bottom). Treatment with Se-TE containing 10.1 or 20.0 μg/kg Se induced a slight rise in the SOD activity in blood, but this enhancement did not happen for selenite or RT + selenite, and a decrease in the SOD activity was found, particularly in the blood. These results indicated that the anticarcinogenic activity of Se-TE may be not due to the effects on their antioxidant activity.

From the hematological studies, it is understood that the significantly higher number of WBCs in tumor groups might be a defensive mechanism against cancer cells. Compared to that of the control (**Figure 4**), the WBC number decreased in

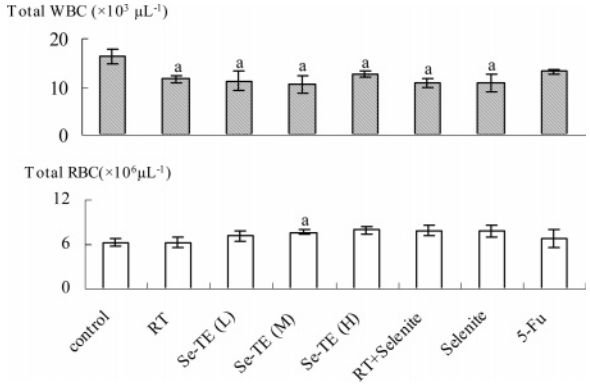

Figure 4. Effect of Se-TE on hematological parameters in tumor-bearing mice. Values are the mean ± SD of nine independent determinations. Letters indicate significant difference: a, different from the corresponding control group, $P < 0.01$.

the mice supplemented with regular tea, selenite, and Se-TEs, suggesting the progression of cancer was brought under control in these mice. All the treatments did not cause a big change in the RBC number of the mice.

DISCUSSION

Though selenium and green tea are both assumed to be cancer-preventive (*27*), the mechanism is not fully understood. The present study was undertaken to investigate whether Se-enriched tea is efficacious in preventing tumor growth in vivo. The tumor nodules in mice produced by injection of HepG2 cells was effectively inhibited by the administration of selenium and regular tea extract. Particularly, both Se-enriched tea extracts were able to give better inhibitory efficiency on tumor growth. This addictive function of Se and green tea extract indicated that the concurrent application of two well-known cancer chemoprevention reagents needs to be explored in the future.

Our results also show that the inhibitory effects of Se-TEs produced from selenite were dependent on the selenium dose. Patients with HCC hepatoma are reported to have a low Se concentration in the blood as well as in the liver (*28*). Here we have found that blood and liver Se increased dramatically by supplementation with Se-TE and selenite (**Figure 2**). However, no difference was found among various Se resources.

It is well documented that selenium and green tea are able to act as antioxidants for biological function. However, in our study intake of selenite and Se-TEs does cause an increase of the activity of the selenium-containing antioxidant enzyme GPx in the blood and liver, but no dose-dependent effect was found, which is consistent with the report that selenoenzyme activity began to reach a plateau after a certain dietary concentration of Se (*29*, *30*). SOD activity in the blood and liver was not affected by supplementation of these selenium resources either. These results favored the understanding that the anticarcinogenic activity of selenium is independent of the function of GPx in the cellular antioxidant systems (*31*) and remaining selenoenzymes (*32*, *33*). It is also reported that green tea polyphenols act as a prooxidant for biological function (*34*). Considering antitumor activity of Se-TE was exhibited at a high concentration of green tea extracts and their prooxidant function, the oxidative status in vivo in mice was not enhanced significantly by administration of tea or selenium. The addition of selenium, especially selenite, will enhance the oxidant activity of green tea extract.

5-Fu is frequently used in the treatment of various types of human cancer. Like other chemotherapy drugs, 5-Fu also exerts undesirable toxic side effects on normal tissues, especially in the gastrointestinal tract (*35*). It was also reported that administration of 5-Fu produced an increase of the lipid peroxide level in the liver and plasma and a decrease of GPx activity in erythrocytes (*10*) and myocardial tissue from guinea pigs (*36*). In this study, we found that mice implanted with HepG-2 hepatoma cells following treatment of 5-Fu had significantly higher GPx activities than those treated with RT and other selenium administrations but still much lower GPx activities than healthy mice without tumor cell inoculation (data not shown), which was in accordance with the previous report that 5-Fu is able to decrease the GPx activities. Meanwhile, 5-Fu provided the highest tumor inhibition effect of 64.1%, while that of Se-TE at a high dose of 20 μg/kg of body weight is 61.8%. Therefore, Se-TE provides us a promising way to screen chemopreventive agents.

In summary, our results demonstrated that supplementation of Se-enriched tea extracts yields better inhibitory effects than regular tea on transplanted hepatocellular carcinoma in mice. Se administration was able to enhance selenium concentrations and GPx activity in the plasma and liver, while Se-TEs did not have significant effects on mouse SOD. This result provides further important insights for screening chemopreventive agents from Se-enriched tea.

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LITERATURE CITED

(1) Diwadkar-Navsariwala, V.; Diamond, A. M. The link between selenium and chemoprevention: a case for selenoproteins. *J. Nutr.* **2004**, *134*, 2899−902.

(2) Ghadirian, P.; Maisonneuve, P.; Perret, C.; Kennedy, G.; Boyle, P.; Krewski, D.; Lacroix, A. A case-control study of toenail selenium and cancer of the breast, colon, and prostate. *Cancer Detect. Prev.* **2000**, *24*, 305−13.

(3) Vogel, V. G.; McPherson, R. S. Dietary epidemiology of colon cancer. *Hematol. Oncol. Clin. North Am.* **1989**, *3*, 35−63.

(4) Sabichi, A. L.; Lee, J. J.; Taylor, R. J.; Thompson, I. M.; Miles, B. J.; Tangen, C. M.; Minasian, L. M.; Pisters, L. L.; Caton, J. R.; Basler, J. W.; Lerner, S. P.; Menter, D. G.; Marshall, J. R.; Crawford, E. D.; Lippman, S. M. Selenium accumulation in prostate tissue during a randomized, controlled short-term trial of l-selenomethionine: a Southwest Oncology Group Study. *Clin. Cancer Res.* **2006**, *12*, 2178−84.

(5) Vanderlelie, J.; Venardos, K.; Perkins, A. V. Selenium deficiency as a model of experimental pre-eclampsia in rats. *Reproduction* **2004**, *128*, 635−41.

(6) Moreno-Reyes, R.; Egrise, D.; Neve, J.; Pasteels, J. L.; Schoutens, A. Selenium deficiency-induced growth retardation is associated with an impaired bone metabolism and osteopenia. *J. Bone Miner. Res.* **2001**, *16*, 1556−63.

(7) Rayman, M. P. Selenium in cancer prevention: a review of the evidence and mechanism of action. *Proc. Nutr. Soc.* **2005**, *64*, 527−42.

(8) Fleet, J. C. Dietary selenium repletion may reduce cancer incidence in people at high risk who live in areas with low soil selenium. *Nutr. Rev.* **1997**, *55*, 277−9.

(9) Popova, N. V. Perinatal selenium exposure decreases spontaneous liver tumorogenesis in CBA mice. *Cancer Lett.* **2002**, *179*, 39−42.

(10) Dong-Li, Chen, K. S. K. C. M. W. Y. T. A. O. Protective effects of selenium supplementation in minimizing 5-fluorouracil induced lipid peroxidative damage of the small intestine. *J. Trace Elem. Exp. Med.* **1997**, *10*, 163−171.

(11) Jiang, C.; Jiang, W.; Ip, C.; Ganther, H.; Lu, J. Selenium-induced inhibition of angiogenesis in mammary cancer at chemopreventive levels of intake. *Mol. Carcinog.* **1999**, *26*, 213−25.
(12) Ip, C.; Lisk, D. J.; Thompson, H. J. Selenium-enriched garlic inhibits the early stage but not the late stage of mammary carcinogenesis. *Carcinogenesis* **1996**, *17*, 1979−82.
(13) Ip, C.; Lisk, D. J.; Stoewsand, G. S. Mammary cancer prevention by regular garlic and selenium-enriched garlic. *Nutr. Cancer* **1992**, *17*, 279−86.
(14) Zeng, H.; Davis, C. D.; Finley, J. W. Effect of selenium-enriched broccoli diet on differential gene expression in min mouse liver-(1,2). *J. Nutr. Biochem.* **2003**, *14*, 227−31.
(15) El-Bayoumy, K.; Richie, J. P., Jr.; Boyiri, T.; Komninou, D.; Prokopczyk, B.; Trushin, N.; Kleinman, W.; Cox, J.; Pittman, B.; Colosimo, S. Influence of selenium-enriched yeast supplementation on biomarkers of oxidative damage and hormone status in healthy adult males: a clinical pilot study. *Cancer Epidemiol. Biomarkers Prev.* **2002**, *11*, 1459−65.
(16) Serafin Munoz, A. H.; Kubachka, K.; Wrobel, K.; Gutierrez Corona, J. F.; Yathavakilla, S. K.; Caruso, J. A.; Wrobel, K. Se-enriched mycelia of Pleurotus ostreatus: distribution of selenium in cell walls and cell membranes/cytosol. *J. Agric. Food Chem.* **2006**, *54*, 3440−4.
(17) Zhao, L.; Zhao, G.; Zhao, Z.; Chen, P.; Tong, J.; Hu, X. Selenium distribution in a Se-enriched mushroom species of the genus Ganoderma. *J. Agric. Food Chem.* **2004**, *52*, 3954−9.
(18) Wang, Z. Y.; Cheng, S. J.; Zhou, Z. C.; Athar, M.; Khan, W. A.; Bickers, D. R.; Mukhtar, H. Antimutagenic activity of green tea polyphenols. *Mutat. Res.* **1989**, *223*, 273−85.
(19) Wang, Z. Y.; Khan, W. A.; Bickers, D. R.; Mukhtar, H. Protection against polycyclic aromatic hydrocarbon-induced skin tumor initiation in mice by green tea polyphenols. *Carcinogenesis* **1989**, *10*, 411−5.
(20) Wang, Z. Y.; Wang, L. D.; Lee, M. J.; Ho, C. T.; Huang, M. T.; Conney, A. H.; Yang, C. S. Inhibition of N-nitrosomethylbenzylamine-induced esophageal tumorigenesis in rats by green and black tea. *Carcinogenesis* **1995**, *16*, 2143−8.
(21) Hu, Q.; Xu, J.; Pang, G. Effect of selenium on the yield and quality of green tea leaves harvested in early spring. *J. Agric. Food Chem.* **2003**, *51*, 3379−81.
(22) Xu, J.; Yang, F.; Chen, L.; Hu, Y.; Hu, Q. Effect of selenium on increasing the antioxident activity of tea leaves harvested during the early spring tea producing season. *J. Agric. Food Chem.* **2003**, *51*, 1081−4.
(23) Amantana, A.; Santana-Rios, G.; Butler, J. A.; Xu, M.; Whanger, P. D.; Dashwood, R. H. Antimutagenic activity of selenium-enriched green tea toward the heterocyclic amine 2-amino-3-methylimidazo[4,5-f]quinoline. *Biol. Trace Elem. Res.* **2002**, *86*, 177−91.
(24) Hu, Q. H.; Pan, G. X.; An, X. X.; Ding, R. L. Physiological function of Se-TE fertilised with sodium selenite and naturally high-Se tea in rats. *J. Sci. Food Agric.* **2001**, *81*, 202−4.
(25) Spolar, M. R.; Schaffer, E. M.; Beelman, R. B.; Milner, J. A. Selenium-enriched Agaricus bisporus mushrooms suppress 7,-12-dimethlybenz[a]anthracene bioactivation in mammary tissue. *Cancer Lett.* **1999**, *138*, 145−50.
(26) Mukhopadhyay-Sardar, S.; Rana, M. P.; Chatterjee, M. Antioxidant associated chemoprevention by selenomethionine in murine tumor model. *Mol. Cell. Biochem.* **2000**, *206*, 17−25.
(27) Peng, S. Q.; Yang, J. S. Effect of deoxynivalenol on action potentials of cultured cardiomyocytes and the protective effects of selenium. *Zhonghua Yufang Yixue Zazhi* **2003**, *37*, 423−5.
(28) Chin-Thin, W.; Wei-Tun, C.; Tzu-Ming, P.; Ren-Tse, W. Blood concentrations of selenium, zinc, iron, copper and calcium in patients with hepatocellular carcinoma. *Clin. Chem. Lab. Med.* **2002**, *40*, 1118−22.
(29) Reeves, P. G.; Leary, P. D.; Gregoire, B. R.; Finley, J. W.; Lindlauf, J. E.; Johnson, L. K. Selenium bioavailability from buckwheat bran in rats fed a modified AIN-93G torula yeast-based diet. *J. Nutr.* **2005**, *135*, 2627−33.
(30) Weiss, S. L.; Evenson, J. K.; Thompson, K. M.; Sunde, R. A. The selenium requirement for glutathione peroxidase mRNA level is half of the selenium requirement for glutathione peroxidase activity in female rats. *J. Nutr.* **1996**, *126*, 2260−7.
(31) Combs, G. F., Jr.; Gray, W. P. Chemopreventive agents: selenium. *Pharmacol. Ther.* **1998**, *79*, 179−92.
(32) Ganther, H.; Ip, C. Thioredoxin reductase activity in rat liver is not affected by supranutritional levels of monomethylated selenium in vivo and is inhibited only by high levels of selenium in vitro. *J. Nutr.* **2001**, *131*, 301−4.
(33) Ganther, H. E. Selenium metabolism, selenoproteins and mechanisms of cancer prevention: complexities with thioredoxin reductase. *Carcinogenesis* **1999**, *20*, 1657−66.
(34) Elbling, L.; Weiss, R. M.; Teufelhofer, O.; Uhl, M.; Knasmueller, S.; Schulte-Hermann, R.; Berger, W.; Mickshe, M. Green tea extract and (−)-epigallocatechin-3-gallate, the major tea catechin, exert oxidant but lack antioxidant activities. *FASEB J.* **2005**, *19*, 807−9.
(35) Davis, L. E.; Lenkinski, R. E.; Shinkwin, M. A.; Kressel, H. Y.; Daly, J. M. The effect of dietary protein depletion on hepatic 5-fluorouracil metabolism. *Cancer* **1993**, *72*, 3715−22.
(36) Durak, I.; Karaayvaz, M.; Kavutcu, M.; Cimen, M. Y.; Kacmaz, M.; Buyukkocak, S.; Ozturk, H. S. Reduced antioxidant defense capacity in myocardial tissue from guinea pigs treated with 5-fluorouracil. *J. Toxicol. Environ. Health, A* **2000**, *59*, 585−9.

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