Nutrition and Cancer

Selenium from High Selenium Broccoli Protects Rats from Colon Cancer[1]

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ABSTRACT Colon cancer is the third most common newly diagnosed cancer in the United States and the third most common cause of cancer-related deaths. Previous supplementation studies have demonstrated the efficacy of selenium (Se) for prevention of colon cancer in humans. The metabolism of Se depends on its chemical form, and studies have shown that the chemical form of Se in broccoli does not accumulate in the body as fast as other forms of Se and may be especially beneficial for prevention of cancer. In the first experiment of the present study, Fisher F-344 rats ($n = 45$) were allotted randomly to torula yeast–based diets supplemented with the following: *1*) no Se; *2*) 0.1 μg Se/g diet as selenate; *3*) 1.0 μg Se/g diet as selenate; *4*) 0.1 μg Se/g diet as selenized broccoli (Se concentration of ~500 μg/g); or *5*) 1.0 μg Se/g diet as selenized broccoli. In Experiment 2, rats ($n = 80$) were allotted randomly to the same basal diet supplemented with the following: *1*) no added Se; *2*) 2.0 μg Se/g diet as selenite; *3*) 2.0 μg Se/g diet as selenite + low Se broccoli; and *4*) 2.0 μg Se/g diet as selenized broccoli. Rats were fed the diets for 2 wk and injected with a chemical carcinogen (3,2 dimethyl 4-amino biphenyl or dimethyl-hydrazine in Experiment 1 or dimethyl hydrazine in Experiment 2; 2 rats/treatment were used as vehicle controls). Supranutritional amounts of Se supplied as high Se broccoli significantly decreased ($P < 0.05$) the incidence of aberrant crypts (AC) and aberrant crypt foci (ACF; preneoplastic lesions indicative of colon cancer) compared with other dietary treatments. Diets were controlled for the presence or absence of broccoli and for the total amount of Se. The reduction in AC and ACF was a function of Se in high Se broccoli and not a result of broccoli alone or Se alone. Adequate dietary Se supplied as high Se broccoli did not accumulate in tissues or increase glutathione peroxidase activity as well as other forms and amounts of Se. Thus, Se from high Se broccoli may be metabolized in a manner that diverts much of the Se into a pool that provides protection against colon cancer. J. Nutr. 130: 2384–2389, 2000.

KEY WORDS: • *selenium* • *broccoli* • *colon cancer* • *selenoprotein* • *rats*

Colon cancer is the third most common newly diagnosed cancer in the United States and the third most common cause of cancer-related deaths (American Cancer Society 1999). About 130,000 people in the United States were diagnosed with colon cancer in 1999 (American Cancer Society 1999). Diet is the single greatest contributor to human cancer, including colon cancer, and may be associated with 35–70% of the incidence of the disease (Doll and Petro 1981). Although various carcinogens are present in foods, their effects are minor compared with dietary components that inhibit the cancer process (Doll and Petro 1981).

Selenium (Se), an essential trace nutrient, has been reported to improve immune function in animals (Beck et al. 1995a and 1995b), enhance neuropsychological function in humans (Finley and Penland 1998) and ameliorate specific disease conditions in humans and animals (Levander 1986). More recently, convincing evidence has been presented that consumption of Se in amounts up of 3–5 times the recommended dietary allowance (RDA;[4] 70 μg/d for men and 55 μg/d women) (National Research Council 1989) may prevent certain cancers including colon cancer. Clark et al. (1996) supplemented human subjects in the Southeastern USA for 10 y with a placebo or 200 μg of Se/d supplied as high Se yeast. Total cancer incidence and mortality were significantly reduced by Se supplementation, with specific reductions of relative risk for lung, prostate and colorectal cancer.

The health benefits of Se, including cancer protection, have prompted an interest in increasing Se intakes beyond the U.S. RDA, but limited choices exist at present for Se supplementation; the most characterized supplement is the high Se yeast that was used by Clark et al. (1996). However, Se also is toxic at intake levels only 5- to 10-fold above supranutritional amounts (Levander 1986), and there is a possibility that supplement abuse, and the resulting increased body burdens of Se,

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[4] Abbreviations used: AC, aberrant crypts; ACF, aberrant crypt foci; DMABP, 3,2′-dimethyl-4-aminobiphenyl; DMH, dimethyl hydrazine; GSH-Px, glutathione peroxidase; GST, glutathione-S-transferase; RDA, recommended dietary allowance; SeMet, selenomethionine; SeMSC, Se-methyl selenocysteine.

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may predispose individuals toward toxicity. In addition, the American Dietetic Association recommends that, whenever possible, people should consume the nutrients they need as foods and not as supplements (Hunt 1996). Therefore, food forms of Se may be preferred over dietary supplements.

Recommending increased intakes of Se through food is complicated by the variability of Se concentrations in foods. Different brand names of the same food product had an almost 10-fold variation in Se content (Finley et al. 1996), probably reflecting different geographical origins of the agricultural commodities used to make the product.

Similarly, the chemical form of Se in foods is variable. Wheat and meat are the most important sources of Se in the North American diet (Holden et al. 1991), and both sources contain large amounts of Se as selenomethionine (SeMet). Selenomethionine is excellent for increasing the Se concentration of tissues (Whanger and Butler 1988), but it is relatively ineffective for suppression of carcinogenesis (Feng et al. 1999, Ip and Ganther 1996). The mechanism for Se inhibition of carcinogenesis is unclear. It is probably not associated with tissue Se accumulation or selenoprotein production, but may be associated with the monomethylated form of Se in the excretory pathway (Vadhanavikit et al. 1993). Consequently, forms of Se that are metabolized preferentially to methyl selenol may provide superior cancer protection (Vadhanavikit et al. 1993).

Se-methyl selenocysteine (SeMSC) is easily converted to methyl selenol and is a primary form of Se found in broccoli and high Se garlic (Cai et al. 1995). Ip and Lisk (1994a, 1994b and 1995) demonstrated that high Se garlic suppresses chemically induced mammary tumors in animals. Garlic consumption is limited by personal preference and social concerns, but broccoli consumption does not have these disadvantages. Consequently, if Se from high Se broccoli proves to have health benefits similar to those of high Se garlic, then high Se broccoli may be an ideal food for increasing the Se intake of humans.

Although colorectal cancer is a form of cancer that was demonstrated by Clark et al. (1996) to be suppressed by Se supplementation, there have been relatively few studies of the effectiveness of nutritional amounts of Se in suppressing the disease. This report uses aberrant crypts (AC) and aberrant crypt foci (ACF) as preneoplastic indicators of colon cancer. Most reports of the effectiveness of Se in preventing AC and ACF have used synthetic forms of Se at pharmacologic doses (Reddy et al. 1996 and 1997); this report concerns the effectiveness of nutritional and supranutritional doses of Se from broccoli.

The objective of this study was to further our understanding of the metabolism and health benefits of Se from broccoli. Specifically, we desired to test the hypothesis that Se from high Se broccoli is better than other forms of Se for suppression of preneoplastic lesions in the colon. Additionally, we wished to study the regulation and distribution of Se from broccoli, and determine whether alterations in these variables may be associated with its unique health benefits.

MATERIALS AND METHODS

Chemicals. 3,2′-Dimethyl-4-aminobiphenyl (DMABP) was purchased from Toronto Research Chemicals (Toronto, Canada). Dimethyl hydrazine (DMH) was purchased from Sigma Chemical (St. Louis, MO). Methylene blue was obtained from Eastman Kodak Company (Rochester, NY).

Production of high Se broccoli. Broccoli (Emperor Hybrid, Northrup King Lawn and Garden, Chattanooga, TN) was planted in one-inch flats containing general purpose growing medium. Seedlings remained in the flats until they developed a root system (~2 wk) and then were transplanted individually into 6.0-L containers. Plants were fertilized with 14–14-14 fertilizer. Approximately 2 wk before heads began to form, 10 mL of 5.2 mmol/L sodium selenate solution was added to each plant container. This solution was added 2 times/wk until the heads became visible; then the amount of solution was increased to 20 mL 2 times/wk until heads were fully formed. Fully formed heads were harvested, immediately frozen and lyophilized. Diets for animal studies used dried powder from the composited heads. Broccoli produced in this manner averaged 500 μg Se/g dry broccoli powder; low Se broccoli was obtained from a local grocery store and averaged <1 μg Se/g dry broccoli.

Animals and diets. All studies were approved by the Animal Care and Use Committee of the Grand Forks Human Nutrition Research Center, and rats were maintained in accordance with the NIH guidelines for the care and use of laboratory animals (NRC 1985).

F-344 inbred rats (weanling males) were purchased from Charles River Laboratories (Wilmington, MA). Rats were housed individually in hanging wire cages in a room controlled for humidity, temperature and light cycle. Rats were given free access to the food and deionized water.

Two experiments were conducted. Experiment 1 used 45 rats allocated to five diets in a manner to equalize initial weights. The basal diet has been described (Davis et al. 1999) and was a low Se torula yeast diet supplied by Tek-Lad (Madison, WI) that was supplemented as follows: low Se broccoli and no added Se (deficient diet), 0.1 mg Se/kg diet as selenate and low Se broccoli (0.1 selenate diet), 1.0 mg Se/kg diet as selenate and low Se broccoli (1.0 selenate diet), 0.1 mg Se/kg diet supplied as high Se broccoli (0.1 broccoli diet) or 1.0 mg Se/kg Se supplied as high Se broccoli (1.0 broccoli diet). All diets contained the same amount of broccoli. If the Se source was not broccoli, or if broccoli that was not as high in Se was needed, the additional broccoli was added as the low Se variety. Seven rats in each group were given injections of carcinogen (see below) and three were injected with vehicle.

Experiment 2 used 80 rats assigned by weight to four diets. The basal diet (same as Experiment 1) was supplemented as follows: no Se and no low Se broccoli (deficient diet); 2.0 mg Se/kg diet as selenite and no low Se broccoli; 2.0 mg Se/kg diet as selenite and low Se broccoli; or 2.0 mg Se/kg diet supplied as high Se broccoli. The last two diets contained equal amounts of broccoli. Eighteen rats in each group were injected with carcinogen (see below) and two were injected with vehicle.

Experimental design. Rats were assigned to treatments and given access to food and water immediately upon arrival. Rats were fed their respective diets for 3 wk and then injected with the carcinogen. The carcinogen used in Experiment 1 was 3,2′-dimethyl-4-aminobiphenyl (DMABP; 100 mg/kg body) in a solution of peanut oil. The carcinogen in Experiment 2 was dimethyl hydrazine (DMH; 25 mg/kg body) in a solution of PBS and 1 mmol/L EDTA. The carcinogen was administered as two subcutaneous injections on consecutive weeks. Details of the administration have been described (Feng et al. 1999). Rats had continuous access to food during the period of injections.

After the second injection, rats were fed their respective diets for an additional 8 wk. Rats were killed by cardiac puncture after ketamine/xylazine anesthesia. Rat tissues were immediately removed, flash frozen in liquid nitrogen and stored at −70°C.

Aberrant crypt analysis. The lower bowel was removed, washed in normal saline, fixed and stored in 70% EtOH until staining and counting of aberrant crypts (AC) and aberrant crypt foci (ACF). The number of ACF gives the number of sites of abnormal colonic cells, whereas the number of AC gives the total number of abnormal cells. The AC and ACF were scored under a dissecting microscope after staining in 0.1% methylene blue by an operator who was unaware of the dietary treatments. Detection and counting of AC and ACF were described previously (Feng et al. 1999).

Selenium status. Selenium concentrations in the plasma and liver were determined by hydride-generation atomic absorption spectrometry according to a previously published procedure (Finley et al. 1996). Samples were prepared for analysis by predigestion in nitric

TABLE 1

Se concentration glutathione-S-transferase (GST) and glutathione peroxidase (GSH-Px) activity of organs and tissues from rats fed a Se-deficient torula yeast diet or a torula yeast diet supplemented with 0.1 or 1.0 μg Se/g diet supplied as selenate or high Se broccoli (Experiment 1)[1,2]

Diet variable	Se deficient	0.1 Selenate	1.0 Selenate	0.1 Broccoli	1.0 Broccoli	$P > F$
Body weight, *g*	314 ± 9.9[a]	321 ± 11.8[a]	309 ± 6.9[a]	309 ± 7.0[a]	309 ± 7.7[a]	0.85
GSH-Px activity, *μ/mg protein*						
Liver	6 ± 2[b]	870 ± 82[a]	864 ± 70[a]	126 ± 8[b]	809 ± 82[a]	0.0001
Muscle	5 ± 0.1[b]	40 ± 4[a]	39 ± 13[a]	45 ± 2[a]	42 ± 14[a]	0.05
Kidney	10 ± 2[b]	400 ± 18[a]	334 ± 54[a]	63 ± 6[b]	321 ± 14[a]	0.0001
Testes	29 ± 8[c]	98 ± 2[ab]	124 ± 9[a]	73 ± 3[b]	96 ± 14[ab]	0.0002
Brain	61 ± 4[b]	111 ± 4[a]	122 ± 1[a]	72 ± 1[b]	112 ± 3[a]	0.0001
Vesicular	105 ± 22[b]	313 ± 18[a]	311 ± 35[a]	241 ± 24[a]	288 ± 38[a]	0.002
Se concentration, *μmol/g*						
RBC	0.14 ± 0.03[d]	7.55 ± 0.20[b]	9.28 ± 0.11[a]	2.61 ± 0.76[c]	7.81 ± 0.60[b]	0.0001
Plasma	0.85 ± 0.11[c]	7.31 ± 0.28[a]	7.83 ± 0.37[a]	3.68 ± 0.15[b]	7.24 ± 0.34[a]	0.0001
Colon	0.92 ± 0.11[c]	3.11 ± 0.06[a]	3.31 ± 0.06[a]	1.81 ± 0.05[b]	2.75 ± 0.32[a]	0.0001
GST activity, *μ/mg protein*						
Liver	494 ± 60[a]	197 ± 66[b]	443 ± 100[ab]	291 ± 20[ab]	267 ± 13[ab]	0.03
Muscle	1 ± 0.10	1 ± 0.04	1 ± 0.03	1 ± 0.02	1 ± 0.10	0.08
Kidney	36 ± 2	31 ± 2	32 ± 2	31 ± 1	31 ± 2	0.40
Testes	252 ± 16	224 ± 6	255 ± 26	283 ± 18	281 ± 10	0.15

[1] Values are means ± SEM; $n = 3$ for GSH-Px and glutathione-S-transferase (GST) values; $n = 9$ for Se concentrations and body weights. Means within a row with different superscripts are different, $P < 0.05$.

[2] Rats were injected with 3,2 dimethyl 4-amino biphenyl (DMABP) on two occasions and fed diets for a total of 12 wk.

acid and hydrogen peroxide, followed by high temperature ashing while in the presence of $MgNO_3$ as an aid to prevent Se volatilization.

Glutathione peroxidase (GSH-Px) enzyme activity was determined by the coupled enzyme method of Paglia and Valentine (1967), which uses hydrogen peroxide as the substrate.

Glutathione-S-transferase. The activity of cytosolic glutathione transferase (GST) was determined spectrophotometrically at 25°C with 1-chloro-2,4-dinitrobenzene as the substrate according to the method of Habig et al. (1974). The reaction mixture contained 100 mmol phosphate buffer (pH 6.5), 1 mmol glutathione and 1 mmol 1-chloro-2,4-dinitrobenzene/L. The reaction was started by the addition of cytosol. Protein concentration was determined by the Bio-Rad protein assay (Hercules, CA).

Statistical analysis. The effect of dietary treatment on animal weights and indicators of Se status was analyzed by one-way ANOVA. If dietary treatment was significant, then individual means were compared by Tukey's pairwise contrasts.

Aberrant crypt data were analyzed by nonparametric statistics by using the generalized linear model procedure (Genmod) in PC/SAS (SAS Institute, Cary, NC). The incidence of aberrant crypts or aberrant crypt foci was assumed to follow a Poisson distribution. For Experiment 1, the independent variables were form and amount of Se. If the overall effect of dietary treatment was significant, then the sources of Se were compared by a priori contrasts nested within the overall design; groups fed Se as selenate (0.1 selenate and 1.0 selenate diets) were compared by χ^2 to groups fed high Se broccoli (0.1 broccoli and 1.0 broccoli diets). For Experiment 2, the independent variable was dietary treatment, which consisted of the control group and the 3 treatment groups. If the overall effect of dietary treatment was significant, then the group fed 2.0 μg Se/g as high Se broccoli was compared by a priori contrasts (χ^2) to each of the other three treatment groups.

RESULTS

Body weights. Dietary treatments did not affect final rat body weights in either experiment (**Tables 1** and **2**).

Experiment 1

Selenium status. Selenium concentrations and GSH-Px activities of most organs and tissues were lowest in rats fed the Se-deficient diet (Table 1). Except for Se-deficient rats, Se concentrations in the muscle and vesicular gland were not affected by dietary treatment. In the liver, kidney and brain, apart from the Se-deficient rats, GSH-Px activity was lowest in rats fed 0.1 mg Se/kg diet as high Se broccoli, and there were

TABLE 2

Se status and body weights of rats fed a Se-deficient torula yeast diet or a torula yeast diet supplemented with 2.0 μg Se/g diet as selenite, 2.0 μg Se/g diet as selenite + low Se broccoli or 2.0 μg Se/g diet as high Se broccoli (Experiment 2)[1,2]

Diet variable	Se deficient	2.0 Selenite	2.0 Selenite + broccoli	2.0 Se from broccoli	$P > F$
Plasma Se, *mmol/L*	0.14 ± 0.03[b]	8.57 ± 0.20[a]	8.82 ± 0.14[a]	8.56 ± 0.14[a]	0.0001
Liver Se, *μmol/g*	0.16 ± 0.01[c]	18.97 ± 0.60[ab]	20.34 ± 0.42[a]	18.57 ± 0.39[b]	0.0001
Body weight, *g*	289 ± 6	295 ± 6	304 ± 5	300 ± 6	0.18

[1] Values are means ± SEM; $n = 20$ for all groups, except group fed 2.0 Se from broccoli, $n = 19$. Means within a row with different superscripts are different, $P < 0.05$.

[2] Rats were injected with dimethyl hydrazine (DMH) on two occasions and fed diets for a total of 12 wk.

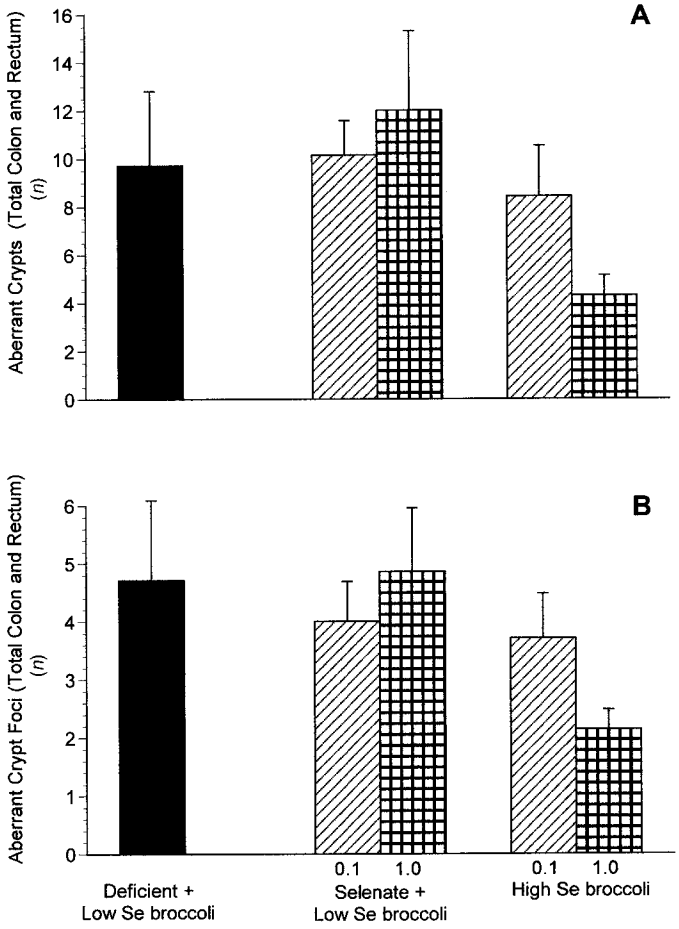

FIGURE 1 Effect of dietary form and amount of Se on the occurrence of preneoplastic lesions in the colons of rats administered 3,2′-dimethyl-4-aminobiphenyl (DMABP) (Experiment 1). Rats were fed diets containing low Se broccoli and no added Se, 0.1 mg/kg Se as selenate and low Se broccoli, 1.0 mg/kg Se as selenate and low Se broccoli, 0.1 mg/kg Se supplied as high Se broccoli or 1.0 mg/kg Se supplied as high Se broccoli. Rats were fed the diets for 3 wk, injected twice with DMABP and then continued to consume the diets for an additional 8 wk. Figures show numbers of aberrant crypts (*A*) and aberrant crypt foci (*B*) in the total colon and rectum. Values are means ± SEM; $n = 7$.

no differences among rats fed 0.1 μg Se/g diet as selenate, 1 μg Se/g diet as selenate and 1 μg Se/g diet as high Se broccoli.

Se accumulation in erythrocytes, plasma and colon was similar to that of GSH-Px, i.e., rats fed the Se-deficient diet had the lowest tissue Se concentrations and rats fed 0.1 μg Se/g diet as high Se broccoli had significantly lower tissue Se concentrations than rats fed other sources or amounts of Se.

GST activity. Liver glutathione transferase activity (Table 1) was significantly lower ($P < 0.03$) in rats fed 0.1 μg Se/g diet as selenate than in those fed the selenium-deficient diet or 1 μg Se/g as selenate. Kidney, muscle and testicular glutathione transferase activities were unaffected by the dietary treatments.

Aberrant crypt and aberrant crypt foci abundance. There was a significant overall effect of diet on the number of AC in rats in Experiment 1 ($P = 0.05$). Nonparametric χ^2 comparisons showed that rats fed diets with Se as high Se broccoli had significantly fewer AC in the total colon and rectum than rats fed Se as selenate ($P = 0.02$; **Fig. 1***A*). There also was a trend ($P = 0.07$) for fewer ACF in rats fed high Se broccoli compared with those fed selenate (Fig. 1*B*). Selenium from broccoli, compared with selenate, significantly reduced the number of AC and ACF in the descending colon (arbitrarily defined as the last one third of the colon and the area of greatest abundance of AC and ACF) (mean; 95% confidence interval: 2.1; 1.4–3.0 and 3.4; 2.6–4.5 for ACF of rats fed broccoli or selenate, respectively, $P = 0.03$; 4.3; 2.7–6.9 and 8.6; 6.2–12.1 for AC of rats fed broccoli or selenate, respectively, $P = 0.02$).

Experiment 2

Selenium status. Dietary treatment significantly altered Se status of rats as judged by differences in plasma and liver Se concentrations (Table 2). Selenium concentrations were lowest in rats fed the Se-deficient diet and highest in rats fed low Se broccoli and 2 μg Se/g diet as selenite. The concentration of Se in the liver of rats fed 2 μg Se/g as high Se broccoli was significantly lower than that in rats fed low Se broccoli and 2 μg Se/g diet as selenite.

Aberrant crypt and aberrant crypt foci abundance. There was a significant overall effect of diet on the formation of AC ($P = 0.01$; **Fig. 2***A*) and ACF ($P = 0.02$; Fig. 2*B*) in the total colon and rectum of rats injected with DMH; χ^2 comparisons of treatment means showed that rats fed 2 μg Se/g

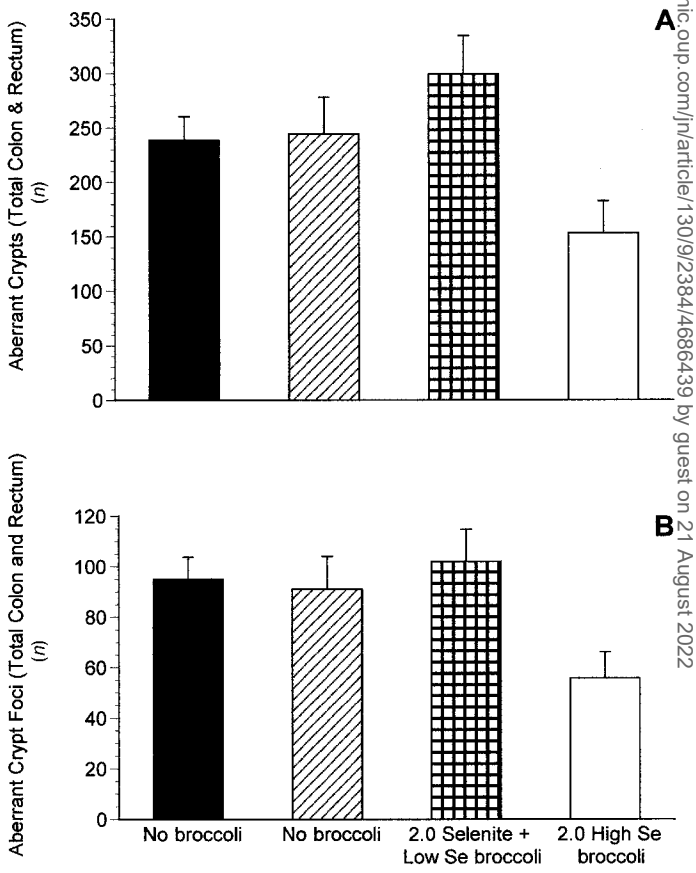

FIGURE 2 Effect of dietary form and amount of Se on the occurrence of preneoplastic lesions in the colons of rats administered dimethyl hydrazine (DMH) (Experiment 2). Rats were fed diets that contained no supplemental Se and no low Se broccoli, 2.0 mg/kg Se as selenite and no low Se broccoli, 2.0 mg/kg Se as selenate and low Se broccoli or 2.0 mg/kg Se supplied as high Se broccoli. Rats were fed the diets for 3 wk, injected twice with DMH and then continued to consume the diets for an additional 8 wk. Figures show numbers of aberrant crypts (*A*) and aberrant crypt foci (*B*) in the total colon and rectum. Values are means ± SEM; $n = 18$.

diet as high Se broccoli had significantly fewer AC (comparison to 0.1 selenite, $P = 0.04$; comparison to 2 selenite, $P = 0.03$; comparison to 2 selenite + low Se broccoli, $P = 0.001$) and ACF (comparison to 0.01 selenite, $P = 0.01$, comparison to 2 selenite, $P = 0.03$; comparison to 2 selenite + low Se broccoli, $P = 0.005$).

DISCUSSION

A recent editorial in a medical journal (Rayman 1997) stated that the available evidence is sufficient to recommend increased Se intakes in Great Britain. Another recently published article (Irion 1999) hypothesized that high Se *Brassica* species may be effective against certain cancers. These reports demonstrate that the medical community recognizes the benefits of increased intakes of Se, but they also recognize that the form of Se that is consumed influences its health benefits. The present research is the first, to our knowledge, to demonstrate that Se from high Se broccoli is superior to salt forms of Se for protection against precancerous lesions in the colon.

Selenium that enters the body may follow one of several metabolic pathways, and that pathway is determined by the chemical form of the Se that was ingested. Selenium consumed as a salt is nonenzymatically reduced to the selenide, which may either incorporate into specific selenoproteins or be methylated sequentially in the excretory pathway. Selenium ingested as SeMet may follow the transsulfuration pathway, be converted to SeCys and then cleaved to produce selenide. However, this is a complex pathway with many regulatory steps, and a more direct fate is for SeMet to substitute for methionine and incorporate into proteins with a methionine requirement (Butler et al. 1989). High Se broccoli, however, contains SeMSC (Cai et al. 1995), which may be converted quickly to methyl selenol by cleavage of the Se-methyl group (Foster et al. 1986). Monomethyl selenol is considered to be the critical metabolite for protection against certain cancers (Ip and Ganther 1996).

In this study, high Se broccoli was more effective than selenate, selenite or broccoli alone for inhibiting preneoplastic lesions in the colons of rats administered either DMABP or DMH. In Experiment 1, 1 μg Se/g diet supplied as high Se broccoli significantly reduced the number of AC in DMABP-treated rats by almost 50%. In Experiment 2, rats fed 2.0 μg Se/g diet as high Se broccoli and injected with DMH (DMH is a more potent carcinogen than DMABP and induces ~20-fold more AC and ACF than DMABP) also had ~50% the number of AC and ACF as rats fed other diets. Both experiments controlled for the total amount of broccoli in the diets, and broccoli alone did not decrease AC or ACF.

The chemical form of Se in broccoli is similar to Se in garlic (Cai et al. 1995). Ip and Lisk (1994a, 1994b and 1995) reported previously that high Se garlic reduced the number of dimethyl benzanthracene–induced mammary tumors in rats. Thus, this study adds to the increasing evidence that Se in the form found in garlic and some *Brassica* species is unique, and especially beneficial for protection against several different cancers. Further, the inhibition of carcinogenesis by high Se broccoli and garlic seems to be a consequence of the uniqueness of Se in those plants, and not a consequence of the total intake of Se, garlic or broccoli.

Because different forms of Se undergo different metabolic transformations, increased anticarcinogenic properties of high Se broccoli must be a result of metabolism that allows more Se to enter the cancer-protective pool. The unique metabolism of Se from high Se garlic and broccoli was reported previously. We fed healthy men stable isotopes of Se as selenate or hydroponically incorporated into broccoli (Finley 1999) and found that Se from broccoli did not accumulate in the plasma as well as selenate. We also repleted Se-deficient rats with 0.1 μg Se/g diets supplied as selenate, selenite, SeMet or high Se broccoli (Finley 1998) and found that Se from broccoli did not accumulate in most tissues and organs to the same degree as other forms of Se, although a few organs accumulated the same amount of Se regardless of dietary source. Ip and Lisk (1994b) reported similar findings with high Se garlic. Rats were fed 3.0 μg Se/g diet as selenite or high Se garlic, and Se concentrations in livers and kidneys of rats consuming high Se garlic were ~70% those of rats fed selenite. However, mammary Se concentrations were similar for high Se garlic and selenite, and muscle Se concentrations were much higher in the group fed high Se garlic. Studies conducted with ^{75}Se-labeled SeMSC (Foster et al. 1986) did not give directly comparable results to studies with high Se garlic and high Se broccoli.

Selenoproteins are primary pools of Se in animals. Consequently, if the body controls the incorporation of Se into selenoproteins, then it may control indirectly the amount of Se that enters cancer-suppressive pathways. In Experiment 1 of this study, rats fed 0.1 μg Se/g diet as selenate, 1.0 μg Se/g diet as selenate and 1.0 μg Se/g diet as high Se broccoli had similar tissue Se concentrations and GSH-Px activities. However, consistent with results from our previous study (Finley 1998), liver, testis, brain and vesicular gland GSH-Px activities, and colon and erythrocyte Se concentrations in rats fed 0.1 μg/g Se as high Se broccoli were $\leq$ 50% of the same measures in rats fed diets supplemented with other forms of Se. The greatest effect of diet was in GSH-Px activity in the liver; rats fed 0.1 μg Se/g diet as high Se broccoli had hepatic enzyme activities that were only 15% of those in rats of the other Se-supplemented groups.

Hepatic GSH-Px is quite labile (Yang et al. 1989) and decreases in times of Se deficiency more readily than other selenoproteins. GSH-Px1 also may act as a Se "buffer" or storage protein (Sunde 1994); decreases in the production of hepatic GSH-Px1 may represent a major diversion of Se into other pathways. When Se from high Se broccoli was fed at a level approximating the nutritional requirement, then this diversion prevented the saturation of GSH-Px activity, a selenoprotein relatively low in the hierarchy for Se use. However, when high Se broccoli supplied 2.0 μg Se/g diet, then there was sufficient Se to maximize GSH-Px activity and protect against carcinogenesis.

The lack of cancer protection by supranutritional amounts of Se in the form of a salt (1.0 mg/kg in Experiment 1, and 2.0 mg/kg in Experiment 2) may be a consequence of unique aspects of the experimental design of this study compared with studies that have found salt forms of Se to be protective (Feng et al. 1999, Ip and Lisk 1994b). Experiment 1 included broccoli in all diets, and it is possible that broccoli alone resulted in changes of AC and ACF formation that were greater than changes induced by selenate. Experiment 2 used 2 μg Se/g diet as selenite and DMH as the carcinogen. Reddy et al. (1996) studied selenite inhibition of azoxymethane, a DMH metabolite, and found a rather modest decrease in ACF in rats with a much greater intake of Se. Rats fed 4.1 μg/g had a mean of 70 ACF/colon, compared with 117 in rats fed 0.1 μg/g Se. Consequently, it is possible that Se as selenite fed at concentrations of 1–2 μg/g has relatively little effect on ACF formation in rats with carcinogenesis induced by DMH and its metabolites.

Selenium "bioavailability" has been assessed traditionally by the ability to replete tissue Se stores and GSH-Px activities. We reported previously that Se from high Se broccoli was less

effective in restoring GSH-Px activity and Se concentrations in most organs and tissues of Se-depleted rats (Finley 1998). By such a criterion, Se from high Se broccoli should be less "bioavailable" than selenate or selenite. Although bioavailablity may confer a specific connotation to many, to others it is used synonymously with "biological usefulness." This study, as well as the studies of mammary cancer and high Se garlic (Ip and Lisk 1994a, 1994b and 1995), demonstrates that the least bioavailable Se compounds may have some of the most potent biological activities. Nutritionists should keep this in mind when dispensing advice on the optimal forms of dietary Se.

In summary, we have demonstrated the Se from high Se broccoli is more effective than selenate or selenite for the prevention of precancerous lesions in the colon of rats. The anticarcinogenic activity of Se in broccoli is caused by the unique chemical form of Se in broccoli. A lesser amount of the Se in broccoli may be used to form selenoproteins, thus allowing more to enter a pool that is protective against colon carcinogenesis.

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