Selenium-Enriched Broccoli Decreases Intestinal Tumorigenesis in Multiple Intestinal Neoplasia Mice

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Cindy D. Davis,1 Huawei Zeng and John W. Finley
U. S. Department of Agriculture, Grand Forks Human Nutrition Research Center, Grand Forks, ND 58202-9034

ABSTRACT Multiple intestinal neoplasia (Min) mice are a good model for the investigation of the effects of dietary alterations in a genetic model for intestinal cancer. Previous studies have shown that selenium-enriched broccoli is protective against chemically induced colon cancer susceptibility. This study investigated whether selenium-enriched broccoli would be protective against intestinal cancer susceptibility in Min mice. Five-week-old heterozygotic male Min mice were fed an AIN-93-based diet containing either low-selenium broccoli or an equivalent amount of high-selenium broccoli for 10 wk. Mice fed the selenium-enriched broccoli had fewer (P < 0.02) small intestinal (46.4 ± 3.7 vs. 65.6 ± 6.1) and large intestinal (0.43 ± 0.17 vs. 1.93 ± 0.27) tumors than those fed an equivalent amount of unenriched broccoli. Min mice fed the selenium-enriched broccoli had small but significant (P < 0.0001) increases in plasma and liver selenium concentrations and red blood cell glutathione peroxidase activity. These results extend previous observations that selenium-enriched broccoli is protective against chemically induced mammary and colon cancer in rats. J. Nutr. 132: 307-309, 2002.

KEY WORDS: • selenium • broccoli • Min mouse • colon cancer

Colorectal cancer is a major human cancer in the United States, accounting for 130,000 new cases and >50,000 deaths each year (1). It is estimated that 50% of the Western population can expect to develop at least one colorectal tumor by the age of 70 yr (2). Selenium, an essential trace element in humans, has received considerable attention for its possible role as a chemopreventive agent. Epidemiologic studies have revealed an inverse association between dietary selenium intake and colon cancer risk in humans (3). A protective role for selenium has also been observed in chemically induced aberrant crypt formation, a preneoplastic lesion for colon cancer, and in colon tumor development (4–7). Human intake of selenium occurs primarily through ingestion of grains and vegetables that contain organic forms of the element such as selenomethionine or selenocystine. Broccoli contains primarily Se-methylselenocystine (8), which is more easily converted to methyl selenol than other organic forms of selenium (9). It has been hypothesized that production of methyl selenol is required for cancer prevention (10). We have recently observed that selenium from selenium-enriched broccoli is more effective than inorganic forms of selenium against chemically induced colonic aberrant crypt formation and mammary cancer development (11,12).

Both human and animal studies suggest that mutation of the tumor suppressor gene adenomatous polyposis coli (APC)4 is a powerful facilitator of intestinal carcinogenesis. Germline mutations of this autosomal dominant gene lead to familial adenomatous polyposis (FAP), a disorder characterized by an early development of multiple adenomas of the colorectum and duodenum with progression to colorectal carcinoma in the third to fourth decade of life in an untreated individual (13,14). Although FAP patients with germline mutations of APC account for <1% of colorectal cancer in the United States, somatic mutations of the APC gene occur in the vast majority of sporadic colorectal cancers (15–17). Such alterations can be found in the smallest lesions examined, such as aberrant crypt foci, suggesting that they are an early event in colorectal tumorigenesis (18,19).

A role for APC in colon carcinogenesis has been further corroborated by the discovery of mouse models of FAP. These models include the multiple intestinal neoplasia (Min) mouse that has a nonsense mutation in codon 850 of the murine APC gene, which is a homolog of the human APC gene (20,21). These mice are highly susceptible to spontaneous formation of numerous tumors in both the small and large intestine (20,21). The Min mouse presents an opportunity to study the pathogenesis of a neoplasm in which the initial genetic defect is the same in the human and mouse. The current study investigated whether selenium-enriched broccoli would be protective against intestinal cancer susceptibility in Min mice.

MATERIALS AND METHODS

Animals and diets. Twenty-eight heterozygotic male Min (C57Bl/6J-ApcMin/+ ApC) mice were obtained at 5 wk of age from Jackson Laboratories (Bar Harbor, ME). All mice were housed individually in a room with controlled humidity, temperature and light. Mice were provided free access to demineralized water and purified diet. The basal diet was an AIN-93 diet (22) containing either low-selenium broccoli or an equivalent amount of high-selenium broccoli. The broccoli was produced as described (11) and accounted for 2.2 g/kg of the diet. By analysis, the diets contained 0.11 and 2.14 Abbreviations used: APC, adenomatous polyposis coli; FAP, familial adenomatous polyposis; Min, multiple intestinal neoplasia.

1 To whom correspondence should be addressed.
E-mail: cdavis@gfhnrc.ars.usda.gov

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mg selenium/kg diet for the control diet and selenobroccoli diets, respectively. Mice consumed their diets for 10 wk.

This study was approved by the Animal Care Committee of the Grand Forks Human Nutrition Research Center, and the mice were maintained in accordance with the guidelines for the care and use of laboratory animals.

**Sample collection.** Mice were deprived of food overnight before killing. After killing, the entire small and large intestines were removed, opened, spread out with the lumen side up and fixed in 10% neutral buffered formalin. The small intestine was divided into three sections of equal length, namely the proximal, middle and distal sections, while the large intestine was not divided. Visible tumors along the entire length of the small and large intestines were counted and measured under a stereo-microscope at a magnification of 20X. Tumor burden was calculated as the sum of the area (length × height) for each tumor in a mouse. One individual, who was unaware of the experimental group, did all of the tumor measurements.

**Selenium status.** Selenium concentrations in the plasma and liver were determined by hydride-generation atomic absorption spectrometry (23). Samples were prepared for analysis by predigestion in nitric acid and hydrogen peroxide, followed by high temperature ashing in the presence of MgNO₃ as an acid to prevent selenium volatilization.

Glutathione peroxidase enzyme activity was determined by the coupled enzyme method of Paglia and Valentine (24) that uses hydrogen peroxide as the substrate.

**Statistical analysis.** The data were analyzed by Student’s t test. Pearson correlations were used to determine the association between tumor burden and weight and indicators of selenium status. All results are expressed as means ± SEM. Differences were considered significant at \( P < 0.05 \).

## RESULTS

Beginning on 25 d of consuming the experimental diets, Min mice fed the selenium-enriched broccoli weighed significantly (\( P < 0.05 \)) more than the mice fed the control diet (Fig. 1). The decrease in weight after 42 d of consuming the experimental diets in the control mice was probably a result of the intestinal tumor burden. Final weight of the mice was significantly correlated with both tumor number (\( r^2 = 0.31 \)) and tumor burden (\( r^2 = 0.34 \)). Furthermore, in control C57BL6 mice fed the same diets, there were no effects of the dietary treatments on body weights at 42 d (23.0 ± 2.6 g vs. 23.2 ± 1.9 g; \( n = 14 \), in mice fed the control and selenobroccoli diets, respectively).

Min mice fed the selenium-enriched broccoli had significantly (\( P < 0.02 \)) fewer small intestinal tumors (Fig. 2) and a smaller (\( P < 0.0001 \)) small intestine total tumor burden than those fed the control diet (Fig. 2). Min mice fed the selenium-enriched broccoli also had a smaller (\( P < 0.002 \)) area/tumor than those fed the control diet (2.93 ± 0.14 vs. 4.37 ± 0.37, respectively). Thus, the individual tumors were smaller in the Min mice fed selenium-enriched broccoli than in mice fed the control diet.

Only a few tumors were found in the large intestine and rectum. However, Min mice fed the selenium-enriched broccoli had fewer (\( P < 0.0001 \)) large intestinal tumors than those fed the control diet (1.93 ± 0.27 vs. 0.43 ± 0.17, respectively).

There were no significant effects of diet on liver glutathione peroxidase activity (**Table 1**). Min mice fed the selenium-

![FIGURE 1](image1.png)

**FIGURE 1** Weight gain in Min mice fed a control diet containing broccoli or a diet containing selenium-enriched broccoli. Values are means ± SEM, \( n = 14 \). Groups differed beginning at d 25, \( P < 0.05 \).

| Table 1: Indicators of selenium status in Min mice fed a control diet containing broccoli or a diet containing selenium-enriched broccoli |
|-----------------|-----------------|-----------------|-----------------|
| Diet            | Selenium concentration | Glutathione peroxidase |
|                 | Plasma (\( \mu \text{mol/L} \)) | Liver (\( \text{nmol/g} \)) | Liver (EU/mg protein) | Red blood cell (EU/mg Hb) |
| Control         | 4.12 ± 0.12      | 41 ± 3          | 1929 ± 133      | 5.15 ± 0.30             |
| Selenobroccoli  | 5.20 ± 0.13*    | 72 ± 2*         | 2229 ± 110      | 9.43 ± 0.38*            |

*Values are means ± SEM, \( n = 14 \). *Different from control, \( P < 0.001 \).

1. Hb, hemoglobin.
enriched broccoli had small but significant ($P < 0.0001$) increases in plasma and liver selenium concentrations and red blood cell glutathione peroxidase activities (Table 1). There was a significant correlation between liver and plasma selenium concentrations and tumor number ($P < 0.02; r^2 = 0.20$), and between liver and plasma selenium concentrations and tumor volume ($P < 0.0002; r^2 = 0.42$). Similar correlations were obtained with red blood cell glutathione peroxidase activity but not with liver glutathione peroxidase activity.

**DISCUSSION**

This is the first study to demonstrate that a food form of selenium, selenium-enriched broccoli, decreases the spontaneous tumorigenesis that occurs in Min mice. This mouse is genetically predisposed to neoplastic lesions associated with the APC gene in the intestine. These results extend our previous observations that selenium-enriched broccoli is protective against chemically induced mammary cancer and aberrant crypt formation (11,12). Similarly, Rao et al. (25) observed that the synthetic selenium compound, 1,4-phenylene bis(methylene)selanocyanate can inhibit both small intestine and colon tumors in Min mice. Our results are similar to the findings of other investigators who have observed that in Min mice most of the tumors usually occur in the small intestine rather than in the large intestine (25,26). However, because of the genetic and histochemical similarities between small intestinal tumors in Min mice and colon cancer in humans, these mice are an accepted model for colon cancer in humans (26). Thus, selenium-enriched broccoli is protective against both chemically induced colon tumor development in rats and spontaneous tumorigenesis in Min mice.

In conclusion, our results demonstrate that selenium-enriched broccoli can decrease the spontaneous tumorigenesis that occurs in Min mice. These results extend previous observations that selenium-enriched broccoli is protective against chemically induced mammary cancer and colon cancer in rats.

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**LITERATURE CITED**