Cruciferous Vegetables and Cancer Prevention

Genoveva Murillo and Rajendra G. Mehta

Abstract: In recent years, cancer prevention by natural products has received considerable attention. The potential protective role of cruciferous vegetables and active components present in these vegetables, such as isothiocyanates and indole-3-carbinol, has been extensively studied in experimental in vitro and in vivo carcinogenesis models. Results have consistently shown that the chemopreventive agents derived from this class of vegetables of the Cruciferae family influence carcinogenesis during initiation and promotion phases of cancer development. Similarly, reports from epidemiological studies and clinical trials support this notion. However, there is no comprehensive summary of all these aspects of the association between cruciferous vegetables and cancer prevention. We have attempted to summarize experimental carcinogenesis studies as well as clinical trials and studies on the mechanism of action of selective chemopreventive agents isolated and identified within these natural products. Results clearly point toward a positive correlation between cancer prevention of many target organs and consumption of cruciferous vegetable or their active constituents. Yet we are still far from complete understanding of the effects of combinations of chemopreventive phytochemicals present in these cruciferous vegetables and their overall mechanism(s) of action in providing protective effects.

Introduction

For more than 25 years, the interdependence between nutrition and the development and progression of cancer has been recognized. The key challenge has been to identify the specific components responsible for contributing to this relationship. In the United States, cancers of the lung, colon and rectum, breast, and prostate account for almost half of the total cancer incidence. In other countries, these types of cancers occur with much less frequency (1). For example, people in China, Japan, and Korea are 4–10 times less likely to be diagnosed with colorectal or breast cancer than are people in the United States (2). Studies have been employed to elucidate the potential factors involved in geographical variation among ethnic groups.

The existence of an etiologic relationship between dietary practices and human cancer is evidenced by the data found when the per capita consumption of specific foods (e.g., dietary fat) is compared with rates of cancer among different countries. Similarly, ecological studies have concluded that the differences in cancer rates are correlated with differences in dietary patterns (1–3). The field of epidemiology has been instrumental in highlighting the relationship between diet and cancer incidence. For example, numerous reports have supported the association between high fat intake and increased incidence of colon cancer (3–7). Similarly, migrant studies have been important in determining the association between cancer rates and dietary habits (8–11). The relationship between migration and cancer has been observed in Polish and Japanese immigrants living in the United States (8,9) and Australia (10) and Indian immigrants living in London (11).

Cumulatively, epidemiological, etiologic, ecological, and migration studies suggest that cancer rates are associated with environmental factors. Because of these studies, numerous investigations have attempted to identify dietary agents that may inhibit the multistage process of carcinogenesis. The field of nutrition and cancer in recent years received a considerable stimulus with the finding of a number of naturally occurring substances that have shown cancer-preventive action in experimental models. Here we review the role of cruciferous vegetables in cancer prevention.

Carcinogenesis and Chemoprevention

The process of carcinogenesis, in which normal cells become malignant, is quite complex. To understand the activities of chemopreventive agents, a brief introduction to the process of carcinogenesis may be useful. With skin carcinogenesis used as a model, it has been established that there are three distinct stages in the development of cancer (12,13). These stages are defined as initiation, promotion, and progression. Initiation is irreversible damage of DNA...
caused by viral, chemical, or physical agents. When this damage affects the function of genes that are involved in cell differentiation, such as suppressor genes, protooncogenes, or transcription factors, it may result in the development of cancer (14). The possible sequence of events during the initiation phase is shown in Fig. 1. Procarcinogens can be eliminated from the body by nonenzymatic inactivation or can form covalent DNA adducts, which in turn may lead to mutation or transformation.

The promotion step is a process by which the initiated cell is transformed into a preneoplastic or neoplastic cell (12). This process is epigenetic and is affected by endogenous and exogenous factors. The process of promotion is accompanied by activation of protooncogenes or inactivation of suppressor genes, which leads to defects in differentiation, growth control, and resistance to host immune response.

The third and final phase of carcinogenesis is known as progression, where the malignant cells become increasingly invasive and metastatic. This is probably due to increased accumulation of genetic abnormalities, increased diversification, and heterogeneity. For example, cumulative evidence from molecular biology and cytogenetic studies shows that most epithelial cancers contain multiple chromosomal deletions and rearrangements. In fact, although there appears to be some unique target-organ specificity, there are similarities in the malignant transformation for most epithelial tissues. For example, increased heterozygosity by loci mapping of chromosome 17 has been reported for lung, breast, and colon, the most common targets for cancer, whereas 3p deletions are associated with kidney and lung tumors in humans. Thus knowledge of the mechanism of each step of carcinogenesis can lead to development of novel approaches to cancer prevention.

Sporn and co-workers (15,16) coined the term “chemoprevention” in 1976, and since then chemoprevention has become a separate discipline of cancer research. Conceptually, chemoprevention of cancer can be defined as an intervention in the carcinogenic process by an agent either naturally derived or synthetic in nature. An agent that blocks, arrests, or reverses the progression of cancer can be termed a chemopreventive agent. In a broad sense, chemoprevention of cancer is a mode of cancer control in which formation of the disease can be prevented. In practice, this can best be achieved by the dietary administration of chemical agents that can enhance the physiological processes that protect the organism against the growth of preneoplastic or cancer cell growth. Because there exists a diversity of mechanisms for inhibition of carcinogenesis, the likelihood that multiple approaches can be made for identifying novel chemopreventive agents is also significantly high. Investigations on the chemoprevention of cancer focus on two categories of a population: the healthy population and individuals who are at a higher risk of developing cancer. Individuals in the general population certainly are exposed to environmental carcinogens and are at risk of developing cancer; however, they are currently disease free. The only attractive way of chemoprevention in healthy people is by intelligent selection of foods (17). On the other hand, people at high risk of developing cancer may reduce the risk by

Figure 1. Evolution stages of neoplasia. ACF, aberrant crypt foci.
selection of chemopreventive foods, as well as by use of chemopreventive agents that may be purely synthetic.

Wattenberg and co-workers (18,19) classified chemopreventive agents into two major categories: 1) blocking agents or anti-initiators and 2) suppressing or antipromotional agents. Some of the blocking agents in food include indoles, isothiocyanates, and thioulethiones in cruciferous vegetables, terpenes from citrus fruits, organosulfurs from garlic, epigallocatechin gallate from green tea and protease inhibitors from beans, as well as some carotenoids, coumarins, and curcumin. On the other hand, antipromotional agents include retinoids, vitamin D analogs, inhibitors of prostaglandin synthesis, monoterpenes, selenium, inhibitors of polyamine biosynthesis, and other agents selectively affecting the post-initiation stage of carcinogenesis. Recent progress in the field of chemoprevention has focused on the identification of molecular targets. On the basis of the mechanism of action of a chemopreventive agent, more efficacious chemicals can be synthesized. Thus the antioxidant properties of chemopreventive agents could serve as carcinogen detoxification agents and be classified as anti-initiators. Similarly, glutathione is a scavenger of carcinogens, and this reaction is catalyzed by glutathione S-transferase. Therefore, glutathione S-transferase or quinone reductase could inhibit carcinogen action. The antipromotional effects of chemopreventive agents involve a variety of molecular targets, affecting numerous signal transduction pathways. These molecular targets include, but are not limited to, hormone receptors, cell cycle check-point markers, transcription factors, mitogen-activated protein kinases, second messengers such as adenosine 3',5'-cyclic monophosphate, rate-limiting enzymes including ornithine decarboxylase, cyclooxygenases, cell junctions, and tumor suppressor genes (e.g., p53). These targets are selective and specific for chemopreventive agents and their action. In the present review, we focus on the current status of cruciferous vegetables in cancer chemoprevention.

**Cruciferous Vegetables**

Cruciferous vegetables are a group of vegetables named for their cross-shaped flowers. Cruciferous vegetables include cabbage, broccoli, Brussels sprouts, cauliflower, and other members of the family Brassicaceae and genus *Brassica* (Table 1). Several reports have demonstrated cruciferous vegetables and/or their constituents to be potentially cancer preventive (20). Isothiocyanates, degradation products of glucosinolates, which occur naturally in a variety of cruciferous vegetables, are some of the most studied components of cruciferous vegetables. More than 20 natural and synthetic isothiocyanates have been studied for their ability to prevent carcinogenesis (21). Volatile isothiocyanates are released from thioglucosides in vegetables and contribute to the distinctive odor and flavor of cruciferous vegetables. Allyl isothiocyanate was identified nearly 50 years ago as a compound particularly prevalent in cabbage. Phenethyl isothiocyanates were found to be abundant in watercress.

### Table 1. Some Members of the Cruciferous Vegetable Group

<table>
<thead>
<tr>
<th>Arugula</th>
<th>Horseradish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beet greens</td>
<td>Kale</td>
</tr>
<tr>
<td>Bok choy</td>
<td>Kohlrabi</td>
</tr>
<tr>
<td>Broccoli</td>
<td>Mustard greens</td>
</tr>
<tr>
<td>Broccoli sprouts</td>
<td>Radishes</td>
</tr>
<tr>
<td>Brussels sprouts</td>
<td>Rutabaga</td>
</tr>
<tr>
<td>Cabbage</td>
<td>Swiss chard</td>
</tr>
<tr>
<td>Collard greens</td>
<td>Turnips</td>
</tr>
<tr>
<td>Garden cress</td>
<td>Turnip greens</td>
</tr>
</tbody>
</table>

(22). Our laboratory has extensively studied the chemopreventive effects of brassinin, an indole present in Chinese cabbage, as a phytoalexin (23,24). Although a variety of chemicals with chemopreventive potential are present in these vegetables, their concentration depends on various factors, including rainfall, sun exposure, soil, and seed stock. Additionally, it has been reported that normal food preparation methods can substantially inactivate isothiocyanate activity (25). Consequently, it is difficult to correlate vegetable consumption and intake of protective compounds. For this reason, attention has focused on using isolated compounds (26) and/or a special seed stock yielding sprouts rich in selective chemopreventive agents, such as isothiocyanates, to ensure adequate levels of the active compound.

**Epidemiological Investigations of Cruciferous Vegetables and Cancer**

The medicinal value of cruciferous vegetables has been reported since ancient times (27). In more recent years, attention has focused on the anticancer effects of cruciferous vegetables. The epidemiological data have been recently reviewed by Verhoeven et al. (28). Of the seven prospective studies, five (71%) reported an inverse association between the consumption of one or more *Brassica* vegetables and cancer risk. Similarly, Verhoeven et al. examined evidence from case-control studies. Consumption of cabbage, broccoli, cauliflower, or Brussels sprouts was inversely associated with risk of cancer in 37 (70%) studies, whereas 11 (20%) studies reported no association and 7 (13%) showed a positive association between cruciferous vegetables and risk of cancer. Cumulatively, these studies provide evidence supporting an anticancer effect of cruciferous vegetables.

More recently, Zhang et al. (29) studied the association between non-Hodgkin’s lymphoma and fruit and vegetable intake. The association was examined among 88,410 women who took part in the Nurses’ Health Cohort Study. Participants who provided dietary information in 1980 were followed for up to 14 yr. During this period, 199 cases of non-Hodgkin’s lymphoma were reported. This study found that higher intake of cruciferous vegetables was associated with a relative risk (RR) of 0.67 for women who consumed five or more servings per week compared with those consuming less than two servings per week.
Similarly, increased consumption of cruciferous vegetables has been reported to be associated with a decreased risk of developing prostate cancer. Cohen et al. (30) examined the association of fruit and vegetable intake and prostate cancer risk among newly diagnosed men residing in the Seattle, WA, area. Cases consisted of men ≥65 yr old who had been newly diagnosed with prostate cancer. Controls were men residing in the same area and of similar age. The results of the study showed that, for cruciferous vegetable intake, adjusted for total vegetable intake and other covariates, the odds ratio for comparison of three or more servings per week with less than one serving per week was 0.59 [95% confidence interval (CI) = 0.39–0.90], with a two-sided P trend = 0.02. These results suggest that high consumption of vegetables, particularly cruciferous vegetables, is associated with a reduced risk of prostate cancer.

The potential benefits of cruciferous vegetables in fighting colon cancer have been summarized in a recent meta-analysis. This study reviewed 20 epidemiological studies in the literature in which the effects of cruciferous vegetable intake on risk for developing colon cancer were examined. By controlling for the effects of overall vegetable intake, it was concluded that cruciferous vegetables themselves confer a separate protective benefit against colon cancer. On the basis of the findings from the meta-analytic study, the authors concluded that, with each 10 g of cruciferous vegetables consumed per day, one could expect an 8% decrease in risk for colorectal cancer (31).

The link between cruciferous vegetables and gene interactions in humans has recently been investigated. London et al. (32) examined the effects of a common deletion polymorphism of glutathione S-transferases M1 and T1 (enzymes responsible for conjugating isothiocyanates, leading to more rapid elimination) on the risk for lung cancer in a population of 18,244 men aged 45–64 yr residing in Shanghai, China. Data were obtained from annual contacts with the surviving members of the cohort and biannual reviews of cancer reports from the Shanghai cancer registry and death certificates. Total isothiocyanate concentrations in the urine were measured before diagnosis. Blood samples were collected to determine glutathione S-transferase M1 and T1 status of 232 incident cases of lung cancer and 710 matched controls selected from the cohort. This study found that individuals with detectable levels of isothiocyanates in the urine had a decreased risk of lung cancer (smoking-adjusted RR = 0.65, 95% CI = 0.43–0.97). However, the greatest protection against lung cancer was observed among individuals with homozygous deletion of glutathione S-transferases M1 (RR = 0.36, 95% CI = 0.20–0.63) and particularly with deletion of glutathione S-transferase M1 and T1 (RR = 0.28, 95% CI = 0.13–0.57). This study supports the use of isothiocyanates to reduce lung cancer risk in humans. Furthermore, this study shows that the effect of isothiocyanates is strongest in people without the gene coding for glutathione S-transferase, in whom isothiocyanate metabolism is predicted to be slower.

Carcinogenesis

Some of the earliest work on cancer chemoprevention by components of cruciferous vegetables was reported by Wattenberg and co-workers (33–35). Treatment with benzyl isothiocyanate 2 h before the carcinogen inhibited 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary carcinogenesis. Bresnick et al. (36) fed cabbage to rats after the N-methyl-N-nitrosourea (MNU)-induced initiation event was completed to evaluate its effects on the promotion phase of mammary carcinogenesis. Results indicated that 5% and 10% dried cabbage in the diet reduced the tumor multiplicity in these animals. However, cabbage was ineffective in the group of rats consuming a high-fat diet. Since then, several groups have studied the effects of synthetic aromatic isothiocyanates to show inhibition of carcinogen-induced mammary, forestomach, and lung carcinogenesis. Chung and colleagues (37) showed that aromatic isothiocyanates are powerful inhibitors of tobacco-specific nitrosamine-induced lung carcinogenesis in rodent models. The experiments to evaluate the structure-activity relationship of isothiocyanate against 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung carcinogenesis have shown that the longer the alkyl chain (up to 6 carbons), the more potent the inhibition. It was shown that 6-phenylhexyl isothiocyanate reduced lung cancer multiplicity by 85% in animal experiments (38). Some data on inhibition of carcinogenesis by cruciferous vegetables are summarized in Table 2. Given the large amount of data available, only a sample of the types of studies will be discussed; several reviews have previously discussed some of these data (21,39–42).

Indole-3-carbinol has been the most studied component of cruciferous vegetables. It has been demonstrated to have chemopreventive activity in several different animal models of carcinogenesis, including mammary gland (43,44), colon (45,46), tongue (47), lung (48), forestomach (35), endometrium (48), cervix (49), skin (50), bone marrow (51), and bladder (52). Grubbs et al. (43) showed that indole-3-carbinol, at 50 or 100 mg/day, was highly effective against DMBA- or MNU-induced rat mammary tumors when administered during the initiation or promotion phase of carcinogenesis. These results showed that indole-3-carbinol can prevent mammary carcinogenesis by direct- and indirect-acting carcinogens. Various animal models have been employed to investigate the effectiveness of indole-3-carbinol against colon cancer (45,46). Guo et al. (45) examined the protective effects of indole-3-carbinol against the development of colonic aberrant crypts in rats. Animals were given 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) at Weeks 3 and 4 of a 16-wk period. Indole-3-carbinol was provided in the diet (0.1%) at initiation, at postinitiation, or continuously throughout the study. The results show that indole-3-carbinol was a potent inhibitor of PhIP-induced aberrant crypt foci during all three stages of carcinogenesis (initiation, postinitiation, and with continuous
feeding of indole-3-carbinol). However, the inhibition of large aberrant crypt foci (≥4 crypt/focus) was only significantly different from the control group when indole-3-carbinol was fed during the initiation phase. These results support the theory that indole-3-carbinol has a protective role against PhIP-induced colon carcinogenesis, with the greatest protection being obtained during the initiation phase.

A transgenic mouse model was used to examine whether the administration of physiological doses of indole-3-carbinol would prevent cervical-vaginal cancer in mice expressing...
ing the transgenes for human papilloma virus type 16 (49). At 4–5 wk of age, virgin mice were implanted subcutaneously with 17β-estradiol (0.125 mg or 0.250 mg per 60-day release) and placed on an AIN-76A diet or an AIN-76A diet enriched with 2,000 ppm indole-3-carbinol. At Week 24, surviving animals were euthanized. Results showed that 19 of the 25 control transgenic mice developed cervical-vaginal cancer within 6 mo, and the remainder of the mice developed dysplasia. In the treatment group, only 5% (2 of 24) of the animals developed cancer, and the rest of the mice exhibited dysplasia or hyperplasia. These findings suggest that indole-3-carbinol is a good chemopreventive candidate against the development of cervical-vaginal cancer associated with human papilloma virus.

Brassinin, an indole-based phytoalexin found naturally in Chinese cabbage, has also been shown to have potential chemopreventive properties in several animal models. In our laboratories, brassinin was shown to significantly induce quinone reductase activity in mammary glands in organ culture and to inhibit skin and mammary carcinogenesis (23,24).

Although indole-3-carbinol has shown great promise in many cancer models, it has also been reported to exhibit adverse promoting effects in a number of animal models. For example, exposure to indole-3-carbinol during the postinitiation (promotion) stage was shown to strongly enhance aflatoxin B1-induced liver tumorigenesis in rainbow trout (53–55), diethylaminoethylamine-induced liver tumorigenesis in newborn or young rats (56), 1,2-dimethylhydrazine-induced colon tumorigenesis in rats (57) and mice (58), N-nitrosodiethylamine-induced pancreas tumorigenesis in hamsters (59), liver and thyroid gland tumorigenesis induced by diethylnitrosamine + MNU + dihydroxy-di-N-propylnitrosamine (60), and MNU-induced mammary carcinogenesis in rats (61). Although several of the earlier studies (53,55) have been criticized for using relatively high dietary doses, other studies (62) have used relatively low dietary doses and still observed significant promutagenic effects by indole-3-carbinol when administered during the promotional phase of carcinogenesis. Thus clinical trials with the aim of investigating the chemopreventive effects of indole-3-carbinol should be approached with caution, until its mechanism(s) of action in enhanced tumor promotion and the relation to human cancer risk are better understood.

**Cruciferous Vegetables and Clinical Studies**

The experimental basis for the use of isothiocyanates in clinical studies was first introduced by Chung et al. (22). In this early work, volunteers were fed a breakfast containing 56 g of watercress, a cruciferous vegetable rich in glucosinatursiin. Urine samples were collected over a 24-h period and analyzed for metabolites. Results derived from high-performance liquid chromatography analysis showed the presence of a compound coeluting with a synthetic N-acetyl conjugate of phenethyl isothiocyanate (PEITC-NAC). These investigations have been followed by several other studies that have examined the fate of isothiocyanates and glucosinolates in humans (63,64). Shapiro et al. (65) investigated the levels of urinary dithiocarbamates to quantify the amounts of cruciferous vegetables consumed by healthy volunteers. Subjects were admitted to the testing facilities and placed on a control diet that excluded all foods containing the compounds of interest. The test vegetable was administered at 7 AM, and breakfast was withheld until 10 AM. Results of this study showed that, in non-smokers, urinary dithiocarbamates were detected only after consumption of cruciferous vegetables and condiments rich in isothiocyanates and/or glucosinolates. Dosing studies revealed that eating horseradish containing graded doses of isothiocyanates led to a rapid excretion of proportionate amounts of urinary dithiocarbamates. Similarly, ingestion of broccoli in which myrosinase was heat inactivated also led to proportionate but lower levels of urinary dithiocarbamates. Thus these studies highlight the importance of myrosinase in the conversion of glucosinolates.

Given that myrosinase is deactivated with heat and that we commonly consume cruciferous vegetables in cooked form, the need to determine whether dietary glucosinolates could be converted to isothiocyanates after being cooked became apparent. To quantify the uptake of isothiocyanates, Getahun and Chung (25) used a urinary marker based on the cyclocondensation product formed by the reaction of isothiocyanates and their conjugates with 1,2-benzenedithiol. Nine volunteers consumed a total of 350 g of cooked watercress in which myrosinase activity was completely deactivated. The amount of glucosinolates ingested by each subject was estimated to be 475 µmol. Twenty-four-hour urinary samples from the volunteers showed that total urinary excretion of conjugated isothiocyanate was 1.2–7.3% of the total amount ingested. In a second experiment, subjects who consumed 150 g of uncooked watercress excreted isothiocyanates in the range of 17.2–77.7% of the total ingested isothiocyanates. These results demonstrate that even when myrosinase is inactivated, some conversion of glucosinolates to isothiocyanates takes place in humans after ingestion of cooked watercress. However, the extent of conversion is considerably less than the amount of uncooked vegetables ingested. Thus normal food preparation (cooking) can substantially inactivate the isothiocyanate-related activity. Consequently, to ensure adequate levels of the potential chemopreventive compounds, the use of supplements may be required to adequately study the true effects of these compounds.

Rosen et al. (66) recently investigated the use of indole-3-carbinol for the treatment of recurrent respiratory papillomatisis, a rare disease characterized by benign papillomatous growths of the aerodigestive mucosa (phase I trial). In this study, 18 patients were treated with oral indole-3-carbinol for ≥18 mo. Outcome measures included a change in papilloma growth rate and the need for surgery after the treatment. Cessation of papilloma growth was found in 33% of the study patients, and they did not require surgery. Six patients had reduced papilloma growth rate, whereas six patients showed no clinical response to indole-3-carbinol. These results sug-
gest that indole-3-carbinol may be beneficial for the treatment of recurrent respiratory papillomatosis (66).

Bell et al. (67) recently conducted a study to examine the use of indole-3-carbinol for the treatment of cervical intraepithelial neoplasia (CIN). Thirty patients with biopsy-proven CIN II-III were randomized, and placebo or indole-3-carbinol was administered orally at 200 or 400 mg/day for 12 wk. All patients underwent colposcopy at the initial visit as well as their visits at Weeks 4, 8, and 12. At the end of the study, none (0 of 10) of the patients in the placebo group had a complete regression of CIN, whereas four of the eight patients in the 200 mg/day arm and four of the nine patients in the 400 mg/day arm had complete regression on the basis of their biopsy at Week 12. The results show an RR of 0.50 (95% CI = 0.25–0.99, P = 0.023) for the 200 mg/day group and 0.55 (95% CI = 0.31–0.99, P = 0.032) for the 400 mg/day group. These data show a statistically significant regression of CIN in patients receiving indole-3-carbinol orally compared with the placebo group.

In addition to naturally derived chemopreventive agents from cruciferous vegetables, attention has also been focused on synthetic chemicals exhibiting properties similar to indoles and thiols present in natural products. One such example is oltipraz [5-(2-pyrazinyl)-4-methyl-1,2-dithiole-3-thione], an agent previously used in humans as an anti-schistosomal agent. Animal studies have demonstrated that oltipraz is a potent inducer of phase II detoxification enzymes and inhibitor of colon cancer (68) and development of mammary lesions in organ culture (69). Human studies have examined the use of oltipraz among individuals at high risk for hepatocellular carcinoma (70) and patients with resected colon polyps (71). Wang et al. (70), in a randomized, placebo-controlled, double-blind phase II chemopreventive study, examined oltipraz’s ability to modulate phase I and phase II enzymes. In the study, 234 healthy adults were randomly assigned to receive 125 mg of oltipraz daily, 500 mg of oltipraz weekly, or a placebo. Phase I and phase II metabolites of aflatoxin B1 were identified and quantified by coupled with mass spectrometry or fluorescence detection. Results showed that 1 mo of supplementation with oltipraz at 500 mg/wk led to a 51% decrease in median levels of phase I metabolite aflatoxin M1 excreted in urine compared with administration of placebo (P = 0.03) but had no effect on phase II metabolites (P = 0.871). By contrast, daily administration of 125 mg of oltipraz resulted in a 2.6-fold increase in the phase II metabolite aflatoxin-mercapturic acid (P = 0.17) but had no effect on excreted aflatoxin M1 levels (P = 0.682). This study shows it is feasible to induce phase I and phase II enzymes with oltipraz. Further studies of dose/schedule and biological end points are necessary.

Mechanisms of Action

Most reports on the antimutagenic activity or chemopreventive activity of cruciferous vegetables have addressed its anti-initiation properties. One principal mechanism by which an anti-initiation agent can exert its effects is inhibition of carcinogen activation. Almost all the carcinogens present in foods require metabolic activation (72). These carcinogens include aflatoxin, nitrosamine, polycyclic hydrocarbons, heterocyclic amines, and hydrazines. In addition, it has been reported that extracts of cauliflower and cabbage interfere with the production of mutagens by nitrosation (73). There is considerable agreement that the active agents include ascorbic acid, cysteine, or other compounds acting as reducing agents. Osawa and co-workers (74) showed that ascorbic acid is responsible for the chemical reduction of the 1,2-dinitro-2-methyl pyrrole, the mutagenic nitrosation product of ascorbic acid, and the nonmutagenic compound 1-nitro-2-methyl-4-amino pyrrole. On the other hand, Stohs and co-workers (75) identified four specific compounds isolated from Savoy chickeftain cabbage that demonstrated antimutagenic activity induced by MNU and 2-aminoanthracene. These compounds, β-sitosterol, pheophytin, nonacosane, and nonacosanone, are notable, especially because they are likely to be present in most plants. These compounds were shown to present different activity profiles against MNU and 2-aminoanthracene; therefore, it was argued that these compounds were achieving their antimutagenicity through more than one biological mechanism.

Another mechanism that has been proposed by Munzner (76) related to the antimutagenic activity of many vegetables, including cabbage, brussels sprouts, and kohlrabi, was the stimulation of the S-9 mix normally used to metabolize, and sometimes activate, mutagens. This observation serves to bridge the antimutagenic potential discussed above and the large body of data that makes it clear that, in animals, there is a strong stimulation of many of the native detoxification systems by extracts of various Brassica species.

The earliest work on the induction of these enzyme systems was actually an attempt by Wattenberg and Loub (35) to explain variations in “baseline” levels of aryl hydrocarbon hydroxylase in different rat colonies. The variation was ultimately ascribed to the presence of alfalfa as an occasional component in rat chow. This observation was followed by an examination of the ability of many foods to stimulate this enzyme. Loub et al. (77) demonstrated that many compounds present in members of the Brassicaceae family (e.g., indole-3-carbinol, 3,3′-diindolylmethane, and indole-3-acetonitrile) were also active in this regard. These compounds significantly induced hydrocarbon hydroxylase activity in the livers of rats consuming augmented basal chow (77).

In subsequent reports, the authors demonstrated that the ability of intestinal enzymes to detoxify many xenobiotic compounds, including the indoles noted above, correlated to brussels sprouts or cabbage consumption in rats and humans. The enzyme systems involved included a number of mixed-function oxidases such as phenacetin O-dealkylase, 7-ethoxycoumarin O-dealkylase, hexobarbital hydroxylase, and benzo[a]pyrene hydroxylase. A direct correlation was later established by McDanell and co-workers (78) between the induction of these activities and the concentration of these compounds. These later studies also demonstrated that the various
active compounds differed in their ability to stimulate enzymes in different organs of the body. They noted, for instance, that the ascorbic acid conjugate of indole-3-carbinol is the most active compound in stimulating the mixed-function oxidase population of the gut, whereas indole-3-carbinol was the strongest inducer of liver enzymes.

Tanaka and colleagues (47) reported the ability of indole-3-carbinol to inhibit tongue carcinogenesis induced with 4-nitroquinoline-1-oxide. Meanwhile, Bradfield and Bjeldanes (79) not only confirmed the earlier results of Loub et al. (77) but also demonstrated that the enzyme glutathione S-transferase was also strongly induced by Brussels sprouts. This enzyme, unlike those discussed earlier, is not a P-450-type enzyme but represents, rather, a phase II detoxification system that acts to conjugate and clear toxicants from the system. The significance of this difference cannot be overstated. For most P-450-type enzymes, their ability to detoxify many mutagens must always be balanced by their ability to activate other mutagens. In the case of glutathione S-transferase, there are no such drawbacks; rather, an increase in this enzyme alone directly resulted in an 87% reduction in the binding of aflatoxin to hepatic DNA in vivo (80). A wide spectrum of compounds, including the glucosinolates, such as sinigrin and progoitrin, and their derivatives, such as allyl-isothiocyanate, goitrin, indole-3-carbinol, and indole-acetonitrile, induce glutathione S-transferase. In other systems, it is induced even more strongly by xanthotoxin and some flavonoids.

In recent years, Talalay and co-workers (81) employed induction of quinone reductase [(NADPH):quinone acceptor] oxidoreductase] as a phase II detoxification biomarker. Using enzyme induction as a parameter, they identified erucin, a sulfide analog of sulforaphane in broccoli. Erucin also induced quinone reductase activity in murine hepatoma cells in culture. However, it was relatively less potent than sulforaphane. Mehta et al. (24) synthesized several analogs of brassinin (a phytoalexin present in Chinese cabbage) and evaluated their ability to induce quinone reductase activity. There is growing evidence suggesting that, aside from the abilities of cruciferous vegetables to inhibit metabolic activation of phase I enzymes and to induce detoxification of carcinogens via phase II enzymes, several other mechanisms may play pivotal roles in the cancer-preventive properties of these compounds. Figure 2 summarizes the mechanisms by which cruciferous vegetables potentially inhibit tumorigenesis.

In estrogen-dependent cancers, such as breast and endometrial cancer, it was first demonstrated that cruciferous vegetables elicit a protective effect via their effects on the estrogen metabolism pathway. Specifically, cruciferous vegetables have been shown to shift production of the estrogen metabolite 16α-hydroxyestrone to the less potent estrogen metabolite 2-hydroxyestrone. This change in estrogen metabolite production has been shown to be protective against estrogen-dependent cancers, because, much like estradiol, 16α-hydroxyestrone has been reported to increase breast cancer proliferation in vitro (82,83) and to promote mammary gland tumors in murine models (83). Unlike 16α-hydroxyestrone, 2-hydroxyestrone has a low affinity for the estrogen receptors and is rapidly methylated by catechol-O-methyl transferase (84), making it less available to the estrogen receptors and, thus, less tumorigenic.

More recently, Meng et al. (85) reported that compounds in cruciferous vegetables are able to modulate estrogen receptor (ER) transcription activity. In this study, a reporter

---

**Figure 2.** Proposed mechanisms of action of cruciferous vegetables.
gene driven by the ER was used to study the effects of indole-3-carbinol on ER-α signaling in human tumor cells. The results showed that indole-3-carbinol (10–125 µmol/l) significantly repressed the 17β-estradiol-activated ER-α signaling in a dose-dependent manner. Furthermore, indole-3-carbinol downregulated expression of the estrogen-responsive genes pS2 and cathepsin-D and upregulated BRCA1. Thus these studies suggest that compounds in cruciferous vegetable may not only affect estrogen metabolism but also function at the transcriptional level.

Meng et al. (86) studied the effects of indole-3-carbinol on cell migration and invasion behavior in ER-positive MCF-7 and ER-negative MDA-MB-468 human breast cancer cell lines. They found that indole-3-carbinol (50 or 100 µM) elicited a significant inhibition in in vitro cell adhesion, migration, and invasion as well as lung metastasis in vivo. Indole-3-carbinol also suppressed the 17β-estradiol-stimulated migration and invasion in estrogen-responsive MCF-7 cells. These findings indicate that the anti-invasion and anti-migration activities of indole-3-carbinol occur via estrogen-independent and estrogen-dependent pathways. Moreover, indole-3-carbinol was found to cause a significant dose-dependent increase in E-cadherin, three major catenins (α, β, and γ), and BRCA1 expression. These findings demonstrate that indole-3-carbinol can activate the function of invasion-suppressor molecules associated with the suppression of invasion and migration in breast cancer cells.

Various reports have suggested that the chemopreventive effects of cruciferous vegetables may be associated with their ability to induce cell cycle arrest in cancerous cells (87,88). Telang and colleagues (87) examined the effects of treating chemically transformed breast epithelial (184-B5/BP-reduction mammaplasty-derived cells) and MDA-MB231 breast epithelial cancer cells with indole-3-carbinol. They found that 50 µM indole-3-carbinol resulted in a 137–210% increase in Q/P ratio (Q = G0, P = S + M) and a twofold increase in cellular apoptosis. Similarly, isothiocyanates have been shown to induce apoptosis in several cancerous cell lines. Chiao et al. (88) investigated the effects of PEITC-NAC on tumor cell growth of human prostate cancer cell lines LNCaP (androgen-dependent) and DU-145 (androgen-independent) cells. This study found that PEITC-NAC arrested cells in the S and G2/M phases of the cell cycle, thus blocking cells from entering replicating phases. Moreover, HeLa cervical cancer cells treated with apoptosis-inducing concentrations of isothiocyanates stimulated an induction of caspase-3/CPP32-like activity, a caspase involved in apoptosis (100). This study suggests that isothiocyanates may induce apoptosis through a caspase-3-dependent mechanism. Thus cruciferous vegetables have multiple modes of actions. As a class, these compounds can mediate their actions as anti-initiators and antipromoters. It is quite remarkable that so many biological activities have been demonstrated for plants as commonly consumed as these plants.

In this review, we have summarized the cancer-preventive potential of many members of the Brassicaceae family. Although the correlation of cruciferous vegetables and prevention of carcinogenesis is strong, it must always be stressed that, to understand the relevance of these reports on the human condition, many further studies need to be done to specifically address questions of the stability, bioavailability, transport, and metabolism. The possible additive or even synergistic effects of these compounds are unknown. The additional effects of normal food preparation procedures present another factor that is yet largely unexplored with respect to the cancer-preventive properties of phytochemicals present in these vegetables.

Acknowledgments and Notes

This work was supported by National Cancer Institute Program Project P01 CA-48112. Address correspondence to Dr. Rajendra Mehta, Dept. of Surgical Oncology (MC/820), University of Illinois College of Medicine, Clinical Science Bldg., 840 S. Wood St., Chicago, IL 60612-7322.

Submitted 26 April 2001; accepted in final form 13 September 2001.

References


26 Nutrition and Cancer 2001


