Significance of Garlic and Its Constituents in Cancer and Cardiovascular Disease

Cancer Chemoprevention by Garlic and Garlic-Containing Sulfur and Selenium Compounds

Karam El-Bayoumy,* Raghu Sinha,* John T. Pinto,† and Richard S. Rivlin**

*Biochemistry and Molecular Biology, Penn State College of Medicine, Hershey, PA 17033; †Laboratory of Molecular Neurobiology, Cornell-Burke Medical Research Institute, White Plains, NY 10605; and **Anne Fisher Nutrition Center, Clinical Nutrition Research Unit, Strang Cancer Prevention Laboratory, Weill Medical College of Cornell University, New York, NY 10021

ABSTRACT As early as 1550 B.C., Egyptians realized the benefits of garlic as a remedy for a variety of diseases. Many epidemiological studies support the protective role of garlic and related allium foods against the development of certain human cancers. Natural garlic and garlic cultivated with selenium fertilization have been shown in laboratory animals to have protective roles in cancer prevention. Certain organoselenium compounds and their sulfur analogs have been identified in plants. Organoselenium compounds synthesized in our laboratory have been compared with their sulfur analogs for chemopreventive efficacy. Diallyl selenide was at least 300-fold more effective than diallyl sulfide in protecting against 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary adenocarcinomas in rats. In addition, benzy l selenocyanate inhibited the development of DMBA-induced mammary adenocarcinomas and azoxymethane-induced colon cancer in rats and benzylpyrene-induced forestomach tumors in mice. The sulfur analog, benzyl thiocyanate, had no effect under the same experimental conditions. Furthermore, we showed that 1,4-phenylenebis(methylene)-selenocyanate, but not its sulfur analog, significantly inhibited DMBA-DNA adduct formation and suppressed DMBA-induced mammary carcinogenesis. Collectively, these results indicate that structurally distinctive organoselenium compounds are superior to their corresponding sulfur analogs in cancer chemoprevention. Additionally, synthetic aromatic selenocyanates are more effective cancer chemopreventive agents than the naturally occurring selenoamino acids. Because plants are capable of utilizing selenium in a manner similar to that in sulfur assimilation pathways, future studies should aim at determining whether, under appropriate conditions, these potent cancer chemopreventive synthetic selenium compounds can be synthesized by garlic and related allium foods.

KEY WORDS: • carcinogenesis • chemoprevention • garlic • selenium • sulfur

In the United States, nearly two-thirds of cancer deaths are estimated to be linked to tobacco use and diet. Dietary factors are believed to account for ~40% of all human neoplasia (1–5). Garlic (Allium sativum) is known to significantly affect human health. As early as 1550 B.C., Egyptians had realized the effects of garlic as a remedy for a variety of diseases (6–8). The health benefits of garlic appear to be true today (9).

Several epidemiological studies suggest that consumption of garlic and related allium foods reduces the risk of certain cancer types, including those of the gastrointestinal tract (10,11). Two independent reports showed a reduced risk of prostate cancer with an increased intake of garlic (12,13). Nevertheless, not all reports support a protective role of garlic in cancer prevention; the role of garlic in breast-cancer risk is an example of the lack of consistency in epidemiological studies (14–16).

Preclinical investigations provide convincing evidence that garlic and related sulfur-containing compounds inhibit carcinogen-
induced tumors in various organs (17,18). As early as 1958, Weisberger and Pensky demonstrated an antiproliferative action of garlic, and follow-up studies further documented the protective effects of garlic against the development of a number of cancers (18,19). Consumption of garlic and related sulfur compounds by laboratory animals (Table 1) reduced carcinogen-induced mammary, colon, esophageal, lung, forestomach, skin, and liver tumors (20–30). These studies also indicated that the oil-soluble compound, diallyl disulfide, was more effective than the water-soluble S-allylcysteine in reducing the incidence and delaying the onset of N-methylnitrosourea-induced mammary tumors (20,31). Collectively, preclinical investigations demonstrate consistently that cancer chemoprevention by garlic and related sulfur compounds is clearly evident and appears to be independent of the organ site or the carcinogen employed.

Most of our common foods, however, contain a very low level of selenium; for example, natural garlic contains <0.05 μg Se/g garlic (32). The selenium content of plants is dependent upon the amount of selenium in the soil. The protective role of selenium is supported by numerous epidemiological studies conducted in the United States and abroad, as well as in preclinical and clinical investigations. Several reviews are available on this topic (33–37).

However, the active form, or metabolite, of selenium that is responsible for cancer prevention in epidemiological studies remains unknown. Our results, and those of others obtained from preclinical studies, indicate that the chemopreventive efficacy of selenium depends not only on the dose, but also on the chemical form in which it is administered (38). The bulk of our current knowledge on the mechanisms of cancer prevention by selenium is based on data from animal experiments and studies conducted using in vitro systems (34,39).

**Sulfur- and selenium-containing compounds from natural sources.** Both animals and humans consume a major portion of their dietary sulfur and selenium in organic forms. As described above, sulfur- and selenium-containing compounds have the capacity to protect against several kinds of cancer development. Plants synthesize the sulfur amino acids and their derivatives from sulfite and sulfate in soil. Similarly, plants are also capable of synthesizing selenoamino acids (e.g., selenomethionine, selenocysteine, selenocystathionine, and Se-methylselenocysteine) from selenite and selenate. Several reports are available on the metabolism of selenium compounds in plants (40–43).

Several selenium-containing compounds present in selenium-enriched foods have been identified (44). Briefly, selenomethionine, the most common organic form of selenium available commercially, is the major form of selenium in selenium-enriched wheat, maize, rice, and yeast. A double-blind, placebo-controlled cancer-prevention trial by Clark et al. (45) showed a 63% reduction in prostate-cancer incidences among men supplemented with selenium-enriched yeast. A follow-up of this study continues to show a marked reduction in the incidence of prostate cancer following selenium supplementation (46,47). The protective effect of selenium-enriched yeast against the development of lung and colon cancer was dependent on baseline plasma selenium levels prior to the intervention. Individuals with lower selenium levels in plasma benefited more than those with normal (nutritional) levels. Although the anticancer mechanisms of selenium-enriched yeast have not been clearly defined, the positive outcome of this trial prompted additional clinical trials in the United States and abroad, such as Selenium and Vitamin E Cancer Prevention Trial (SELECT), Prevention of Cancer by Intervention with Selenium (PRECISE), and Australian Prostate Cancer Prevention Trial Using Selenium (APPOSE) (48–50). The major form of selenium in selenium-enriched garlic, onions, broccoli florets, broccoli sprouts, and wild leeks is Se-methylselenocysteine (44).

The chemical and physical properties of selenium are markedly similar to those of sulfur (44). Five oxidation states of selenium are known: −2, 0, +2, +4, and +6. Elemental selenium (Se0) is biologically inert, while selenite (Se2−) and selenate (Se4−) are biologically active forms. Two features that distinguish selenium from sulfur in biological systems are as follows:

### Table 1

<table>
<thead>
<tr>
<th>Carcinogen</th>
<th>Animals</th>
<th>Targets</th>
<th>Literature cited</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-methylnitrosourea</td>
<td>Female SD-rats</td>
<td>Mammary</td>
<td>(20)</td>
</tr>
<tr>
<td>7,12-dimethylbenz[a]anthracene</td>
<td>Female SD-rats</td>
<td>Mammary</td>
<td>(21)</td>
</tr>
<tr>
<td>2-amino-1-methyl-6Phenylimidazo[4,5-b] pyridine</td>
<td>Female SD-rats</td>
<td>Mammary</td>
<td>(22)</td>
</tr>
<tr>
<td>1,2-dimethylhydrazine</td>
<td>Female C57NL/6J mice</td>
<td>Colon</td>
<td>(23)</td>
</tr>
<tr>
<td>N-nitrosomethylbenzylamine</td>
<td>Male SD-rats</td>
<td>Esophagus</td>
<td>(24)</td>
</tr>
<tr>
<td>4-(methylnitrosamo)-1-(3-pyridyl)-1-butane</td>
<td>Female A/J mice</td>
<td>Lung</td>
<td>(25)</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>Female A/J mice</td>
<td>Lung &amp; forestomach</td>
<td>(26)</td>
</tr>
<tr>
<td>N-nitrosodiethyamine</td>
<td>Female A/J mice</td>
<td>Forestomach</td>
<td>(27)</td>
</tr>
<tr>
<td>7,12-dimethylbenz[a]anthracene</td>
<td>Female Ha/ICR mice</td>
<td>Skin</td>
<td>(28)</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>Female SA-mice</td>
<td>Skin</td>
<td>(29)</td>
</tr>
<tr>
<td>1,2-dimethylhydrazine</td>
<td>Male F344 rats</td>
<td>Liver</td>
<td>(30)</td>
</tr>
</tbody>
</table>

SD = Sprague-Dawley; SA = Swiss albino.
follows: 1) selenium compounds are metabolized to more reduced states, but sulfur compounds are metabolized to more oxidized states; 2) the hydride derived from selenium (H2Se) is much more acidic than that derived from sulfur (H2S); i.e., selenium is a good donor of hydrogen ions and is completely ionized at normal blood pH levels.

Comparison of sulfur and selenium analogs in cancer prevention. In view of the similarity between the two elements, it was our goal to develop novel synthetic organoselenium analogs of established sulfur-containing chemopreventive agents to obtain compounds with optimal chemopreventive potency and low toxicity. The rationale for synthesizing these selenium compounds is based on the following concepts: 1) although the known inhibitors of carcinogen-induced neoplasia possess few common structural features, many have functional groups containing either oxygen, sulfur, or selenium, all of which are elements of group VI of the periodic table; 2) replacement of oxygen and/or sulfur by selenium is known to modify the biological activity and therapeutic index of certain drugs, a phenomenon known as isosteric effect in drug design. Accordingly, we substituted selenium for oxygen and/or sulfur in known inhibitors of chemical carcinogenesis. The chemopreventive agents were examined in several animal models and, in some instances, were compared with the historical inorganic selenite, oxygen, and sulfur analogs (51).

To determine whether the substitution of sulfur with selenium in diallyl sulfide would result in a more potent chemopreventive agent, we synthesized diallyl selenide after modification of the method described earlier (52) and compared its efficacy with that of diallyl sulfide in the 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary carcinogenesis model (53). Rats were gavaged 3 times with diallyl selenide (6 or 12 μmol/kg body wt) or diallyl sulfide (300, 900, or 1800 μmol/kg body wt) at 96, 48, and 24 h before DMBA treatment. Both doses of diallyl selenide showed significant tumor inhibition, but only the highest dose of diallyl sulfide showed inhibition. Based on these results, diallyl selenide appeared to be at least 300 times more active than diallyl sulfide.

Volatile garlic sulfides, as exemplified by diallyl sulfide, have reportedly mediated activities of cytochrome P450 isozymes, blocked carcinogen activation, and facilitated carcinogen detoxification through increased activities of glutathione-S-transferase, epoxide hydrolase, and UDP-glucuronosyl transferase, as well as through induction of glutathione peroxidase. The latter effect increases the ratio of glutathione to glutathione disulfide. Collectively, these results can account for cancer chemoprevention by diallyl sulfide in various target organs, including the mammary gland (24, 53–61). In line with the effect of diallyl sulfide on the above-mentioned biochemical parameters, Amagase et al. (62) showed that garlic, as a whole, suppresses the formation of DMBA-DNA adducts in the mammary gland in vivo; certain dietary components can modulate this effect further.

Based on these results, we conducted a biochemical investigation to determine whether diallyl selenide exerts effects similar to those of diallyl sulfide. We examined the effect of diallyl selenide on the initiation phase of carcinogenesis, i.e., DMBA-DNA adduct formation in rat mammary glands. Analysis of total binding and individual DNA adducts in the mammary gland and liver showed that diallyl selenide had no effect on these parameters, suggesting that it might influence critical events in carcinogenesis other than carcinogen activation and/or detoxification. Thus, further research is needed to evaluate whether diallyl selenide is more effective than diallyl sulfide in eliciting responses such as the inhibition of cell proliferation, diminishing ornithine decarboxylase activity, and/or induction of apoptosis (18, 63, 64).

To further support our concept that replacing sulfur with selenium in known chemopreventive agents will result in more effective analogs, we synthesized and compared benzyl selenocyanate (BSC) with benzyl thiocyanate (BTC) in several carcinogen-induced tumor animal models (33). Briefly, we demonstrated that BSC is superior to BTC as an inhibitor of benzo[a]pyrene-induced forestomach tumors in mice, as well as DMBA-induced mammary tumors and azoxymethane-induced colon tumors in rats (51, 65, 66). The results of numerous studies conducted in our laboratory, as well as in others, clearly support the concept described above.

In short, selenium-enriched garlic, selenium-enriched yeast, and selenium-enriched broccoli are each more effective cancer chemopreventive agents in various animal models than regular garlic, yeast, and broccoli, respectively (44). In addition to diallyl selenide and BSC, comparative chemopreventive efficacy of selenocystamine, selenobetaine, and 1,4-phenylenedihydriodiselenium (methylene) selenocyanate with their sulfur-containing analogs in vivo has been reported (37). Collectively, the outcome of these studies (Table 2) led to the conclusion that selenium-enriched food items and selenium compounds are more effective than their corresponding sulfur analogs, respectively (21, 67–69).

| Table 2 |

<table>
<thead>
<tr>
<th>Selenium compounds</th>
<th>Sulfur compounds</th>
<th>Literature cited</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se-Enriched garlic</td>
<td>Regular garlic</td>
<td>(21)</td>
</tr>
<tr>
<td>Se-Enriched yeast</td>
<td>Regular yeast</td>
<td>(67)</td>
</tr>
<tr>
<td>Se-Enriched broccoli</td>
<td>Regular broccoli</td>
<td>(68)</td>
</tr>
<tr>
<td>Selenocystamine</td>
<td>Cystamine</td>
<td>(69)</td>
</tr>
<tr>
<td>HSeCH2CH2NH2</td>
<td>HSCONH2</td>
<td>(69)</td>
</tr>
<tr>
<td>S-methylselenocysteine</td>
<td>S-methylcysteine</td>
<td>CH2SeCH2CHOOH</td>
</tr>
<tr>
<td>CH2SeCH2CHOOH</td>
<td>CH2SeCH2CHOOH</td>
<td>(69)</td>
</tr>
<tr>
<td>NH2</td>
<td>NH2</td>
<td></td>
</tr>
<tr>
<td>Selenobetaine</td>
<td>Sulfobetaine</td>
<td>(69)</td>
</tr>
<tr>
<td>(CH3)2SeCH2COOH,Cl</td>
<td>(CH3)2SeCH2COOH,Cl</td>
<td>(69)</td>
</tr>
<tr>
<td>Diallyl selenide</td>
<td>Diallyl sulfide</td>
<td>(53)</td>
</tr>
<tr>
<td>(CH2)2CHSe2</td>
<td>(CH2)2CHSe2</td>
<td>(65)</td>
</tr>
<tr>
<td>Benzyl selenocyanate</td>
<td>Benzyl thiocyanate</td>
<td>(37)</td>
</tr>
<tr>
<td>C2H5SeCN</td>
<td>C2H5SeCN</td>
<td>(37)</td>
</tr>
<tr>
<td>1,4-phenylenedihydriodiselenium (methylene)</td>
<td>1,4-phenylenedihydriodiselenium (methylene) thiocyanate</td>
<td>(37)</td>
</tr>
<tr>
<td>C2H5(CHO2SeCN)2</td>
<td>C2H5(CHO2SeCN)2</td>
<td>(37)</td>
</tr>
</tbody>
</table>

*Abbreviations used: BSC, benzyl selenocyanate; BTC, benzyl thiocyanate; DMBA, 7,12-dimethylbenz[a]anthracene; ED50, 50% inhibition; MTD, maximum tolerable dose, p-XSC, 1,4-phenylenedihydriodiselenium; p-XSeSG, glutathione conjugate of p-XSC; p-XSeH, aromatic selenol of p-XSC; TSC, tetraselenocyclophane.

Collectively, these studies demonstrate that selenium compounds are superior cancer chemopreventive agents to their corresponding sulfur compounds.
index (70). The chemopreventive index of 1,4-phenylenebis-(methylene)selenocyanate (p-XSC) was the highest, followed by Se-methylselenocysteine, and then selenomethionine, with the lowest index; the latter was comparable to that of the inorganic sodium selenite. Further studies in our laboratories demonstrated that p-XSC is a powerful chemopreventive agent against the development of experimental colon, lung, and oral carcinogenesis (71). We do not yet know whether plants can naturally synthesize these potent synthetic organoselenium compounds. To our knowledge, no studies have reported chemopreventive indices in an animal model of various chemopreventive sulfur-compounds. Because p-XSC had the highest chemopreventive index in rat mammary tumors, the remainder of this manuscript discusses its mechanisms of action.

**The chemical form of selenium is critical in chemoprevention.** Our results, as well as those of others, indicate that not only is the dose important in cancer chemoprevention, but so also is the form of selenium; this suggests that the metabolism of selenium compounds is a prerequisite for cancer prevention (38). After intestinal absorption, dietary selenite is reduced by thiols (e.g., glutathione) and NADPH-dependent reductase, through selenodiglutathione to highly toxic H₂Se. In turn, H₂Se, is converted to selenophosphate, and then incorporated as selenocysteine into numerous selenoproteins, such as glutathione peroxidase. Selenium-containing enzymes do not appear to be as important as selenium metabolites in cancer chemoprevention (34,38).

Extensive studies have concluded that selenium compounds directly converted to mono-methylated forms, (methylselenol, CH₃SeH) or related intermediates (e.g., aromatic selenol) are powerful chemopreventive agents; however, the mechanism that accounts for the role of selenol intermediates in cancer chemoprevention remains undefined.

In previous studies, we synthesized and examined the excretion profile of [³⁵S]XSC in rats and mice in order to gain insights into its mechanism of action (72,73). In rats, feces are the major route of selenium excretion, and in mice, levels of selenium in feces and urine are comparable. However, in both rats and mice, according to selenium measurements, <1% of the dose was detected in exhaled air. Such a low level of the highly toxic H₂Se (measured as CH₃SeCH₃), in this case, indicates that toxicity can be dissociated from chemopreventive efficacy by structural design of appropriate organoselenium compounds. In both species, we also identified tetraselenocyclophane (TSC) in fecal excretion, which led us to postulate the following metabolic pathway: p-XSC → glutathione conjugate (p-XSeSG) → aromatic selenol (p-XSeH) → TSC.

We believe that the formation of the aromatic selenol p-XSeH is an important intermediate in cancer chemoprevention by p-XSC. Because selenols are unstable and thus difficult to prepare, we hypothesized that p-XSeSG would be a more effective cancer chemopreventive agent than p-XSC. Therefore, we synthesized p-XSeSG and compared its efficacy with that of p-XSC in rat mammary tumors using DMBA as the carcinogen.

**Molecular chemoprevention by p-XSC and p-XSeSG: A genomic approach.** To test the above-mentioned hypothesis and to understand the molecular mechanisms that account for cancer prevention by both selenium compounds, the following investigations were conducted. Details of the mammary-cancer bioassay were described previously (74). We showed that when rats were fed continuously, p-XSC was significantly more effective than p-XSeSG. When rats were fed from 1 wk after DMBA to termination (postinitiation phase of carcinogenesis), both selenium compounds were equally effective. We also showed that both selenium compounds significantly inhibited DMBA-DNA adduct formation (75).

These results can account, at least in part, for the mechanism of action of selenium compounds during the initiation phase of carcinogenesis. By contrast, as described above, diallyl selenide had no effect on DMBA-DNA adduct formation. Therefore, selenium compounds, depending on their structure, appear to inhibit carcinogenesis through different mechanisms. Inhibition of cell proliferation and induction of apoptosis, generally, are considered critical cellular events in cancer chemoprevention, especially by selenium compounds during the post-initiation phase of carcinogenesis. Therefore, using a rat mammary cancer cell line, we found that, depending on selenium dose and time point selected, p-XSC is comparable to, or better than, p-XSeSG in inhibiting cell growth. These results suggest that intermediates other than p-XSeSG could be responsible for chemoprevention by p-XSC. Future investigations will focus on validating this hypothesis.

To provide basic knowledge at the molecular level of the efficacy of both selenium compounds, a follow-up study was done using cDNA microarray analysis, followed by quantitative RT-PCR in mammary adenocarcinomas isolated from rats treated with DMBA and control diet, or diets supplemented with p-XSC or p-XSeSG (74,76). Both selenium compounds significantly inhibited various genes related to cytochrome P450 isoforms while they induced the expression of several genes related to phase II enzymes, as well as significantly and equally upregulating p27, p21 and BAD genes. We found that p-XSC was notably more effective than p-XSeSG in the upregulation of proapoptotic genes APO-1 and caspase-3. Both compounds were equally effective in downregulating expression of cyclin D1, cyclin D2, c-myc, and proliferating cellular nuclear antigen genes.

Together, these results show that selenium has an impact on genes involved in the multistep process of carcinogenesis. On the other hand, one could argue that the molecular analysis of adenocarcinomas, which represent the “clones” of cells that escaped chemoprevention by selenium compounds, may not truly reflect the action that accounts for cancer chemoprevention by both compounds. Future molecular analysis, therefore, will be performed on mammary epithelial cells at several stages of carcinogenesis. In addition, the effect of selenium alone (without DMBA) on differential gene expression in normal mammary epithelial cells needs to be determined. The identification and characterization of protein patterns and biochemical pathways involved in tumorigenesis using a “proteomics” approach would also provide better insights, because levels of transcripts using cDNA microarray analysis do not always equate to differences in levels of proteins, or their functional activities. Selenium compounds possess much greater oxidoreductive potentials than sulfur compounds (77) and can cause oxidation of sulfhydryl coordination moieties in many transcription factors and signaling proteins that regulate proliferative and/or apoptotic responses (38).

**DISCUSSION**

With a few exceptions, the chemical and physical properties of selenium and sulfur are similar; both elements belong to group VI of the periodic table. Plants synthesize sulfur amino acids from sulfite and sulfate; similarly, they also synthesize selenoamino acids from selenite and selenate. Data on the possible synthesis of the nonprotein selenoamino acids by plants are lacking. The selenium content of plants is dependent upon the amount of selenium in the soil. Most of our common foods, including garlic, contain a very low level of selenium. Humans consume a substantial portion of their dietary sulfur and...
selenium in organic forms. Several studies in our laboratories and others show that representative examples of synthetic and naturally occurring organoselenium cancer chemopreventive agents are superior to their corresponding sulfur analogs, at least in the rat mammary tumor model. Furthermore, studies in other laboratories demonstrate that selenium-enriched foods, such as garlic, broccoli, and wheat, are more effective than the corresponding regular dietary items.

Among the synthetic organoselenium compounds, p-XSC is the most effective cancer chemopreventive agent in the rat mammary model; p-XSC is also a powerful chemopreventive agent against the development of experimental colon, lung, and oral carcinogenesis. In rat mammary tumors, evidence indicates that methyl selenol is the most active species in cancer prevention by selenium compounds. Similarly, an aromatic selenol (p-XSeH) derived from p-XSC may be the active intermediate against mammary tumor formation, although questions remain regarding how and why selenol intermediates are critical in cancer prevention. Genomics and proteomics approaches will help provide insights on the role of selenium and sulfur-containing compounds in cancer chemoprevention (78). Selenium compounds, depending on their form (structure), inhibit carcinogenesis through different mechanisms.

A stepwise approach must be taken to elucidate how dietary modifications and chemoprevention can be harnessed effectively for cancer prevention and control. Thus, we will continue to search for optimal diets and naturally occurring chemopreventive agents in routinely consumed foods. Future studies should also focus on structural modification of established, as well as naturally occurring, chemopreventive agents that may lead to synthetic agents with even greater chemopreventive indices. The results described here suggest that molecular targets modulated by selenium- and sulfur-containing compounds are highly useful indicators of success in clinical chemocancer-prevention trials. To develop appropriate strategies for cancer chemoprevention, our view is that a cocktail approach (using more than one chemopreventive agent with different modes of action, such as Se-methylselenocysteine or p-XSC, in combination with diallyl sulfide) may be the most practical; future studies in this area are urgently needed.

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LITERATURE CITED
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