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Chemoprevention of Cancer by Organoselenium Compounds

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Abstract A major research goal of our laboratories is the development of new organoselenium cancer chemopreventive agents with less toxicity compared to some of the historical selenium compounds, such as sodium selenite. Ideally, such agents would be employed to inhibit tumor development in different organs caused by a variety of chemical carcinogens, particularly those present in the human environment. A series of organoselenium compounds has been synthesized and evaluated for their chemopreventive efficacy *in vivo*. Parallel to these studies, short-term *in vitro* and *in vivo* assays were employed to understand the mechanism of action and to rapidly evaluate their efficacy in eventual long-term preclinical investigations. We demonstrated that one of the most effective of these organoselenium compounds, 1,4-phenylenebis(methylene)selenocyanate (*p*-XSC, Fig. 1), is capable of inhibiting tumors in the mammary glands, colon, and lung of laboratory animals.

Dietary *p*-XSC inhibited mammary tumor development induced by 7,12-dimethylbenz(*a*)anthracene (DMBA) during both the initiation and post-initiation phases of carcinogenesis in female CD rats. *p*-XSC inhibited DMBA-DNA adduct formation in the mammary glands. In collaboration with other laboratories, we demonstrated that *p*-XSC inhibited thymidine kinase in mammary tumor cell lines derived from both humans and rats. Employing mammary carcinoma cell lines, *p*-XSC was also shown to inhibit cell growth and induce a dose-dependent increase in cell death by apoptosis. In these assays *p*-XSC appears superior to selenite and to its sulfur analog, 1,4-phenylenebis(methylene)thiocyanate. Dietary *p*-XSC decreased colon tumor induction by azoxymethane in F344 rats during both phases of carcinogenesis. The effect of *p*-XSC on colonic aberrant crypt multiplicity showed a similar trend. Colonic mucosal selenium-dependent glutathione peroxidase activity was increased, and prostaglandin E_2 was reduced in animals fed the *p*-XSC diet compared to animals fed the control diet. Dietary *p*-XSC inhibited the formation of DNA adducts, as well as lung tumor development by the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in A/J mice, while selenite had no effect. These observations are important because smokers are exposed to NNK and could conceivably be protected against tumorigenesis by dietary supplements of effective organoselenium compounds. Collectively, these results indicate that *p*-XSC and similar organoselenium compounds are capable of inhibiting tumors in mammary gland, colon, and lung of animals in model systems. Moreover, the results of short-term bioassays described above are encouraging and could assist in the design of even better and less toxic organoselenium chemopreventive agents than *p*-XSC for future application in preclinical assays and subsequently in human clinical intervention.

Key words: Chemoprevention, colon, lung, mammary, organoselenium

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Chemoprevention refers to the administration of chemical agents, either synthetic or naturally occurring, to reduce or prevent the initiating (mutational) and/or promotional events that occur during the process of neoplastic development [1]. The list of agents with chemopreventive ac-

tivity is growing rapidly [2]. One important class of chemopreventive compounds is comprised of organic and inorganic forms of selenium [3].

Increasing epidemiological evidence shows a protective role of selenium in human cancers. In general, the data suggest that selenium plays a role in reducing human cancers, particularly in relation to gastrointestinal tract and respiratory system; however, the available evidence lacks consistency in regard to the role of selenium in breast cancer prevention [4–10].

At doses well above the physiological requirement, selenium is a proven chemopreventive agent in many animal models; supplementing the diet or drinking water with inorganic selenium protects against cancer of the mammary gland, colon, lung, pancreas, liver, and skin [3]. Although inorganic selenium compounds inhibit carcinogenesis, they are toxic. Naturally occurring selenium-containing amino acids such as selenomethionine and selenocysteine were not much more effective than inorganic selenium in cancer prevention and had comparable toxicity [3].

Chronic feeding of inorganic selenium compounds at levels >5 ppm is toxic. Toxic effects of selenium have been described in animals and humans, the liver being a key target [11]. This toxicity inhibited research until the recent introduction of novel synthetic organoselenium compounds [3]. In our laboratory, achieving optimal chemopreventive potency with low toxicity continues to be a priority in the development of organoselenium chemopreventive agents.

We were the first to report use of synthetic organoselenium compounds as chemopreventive agents in laboratory animals [3]. The rationale for synthesizing these agents was based on the following concepts: although the known inhibitors of chemically induced neoplasia appear to possess few common structural features, many of them have functional groups containing either oxygen or sulfur, which are elements of Group VIB of the periodic table, and replacement of oxygen and/or sulfur by selenium is known to enhance the biological activity of certain drugs. For example, it has been shown that selenoguanine inhibits the growth of several experimental tumors, is less toxic, and shows a better therapeutic index than its sulfur analog, thioguanine [12]. The antibacterial activity of 6-selenopurine against 10 types of bacteria exceeded that of 6-

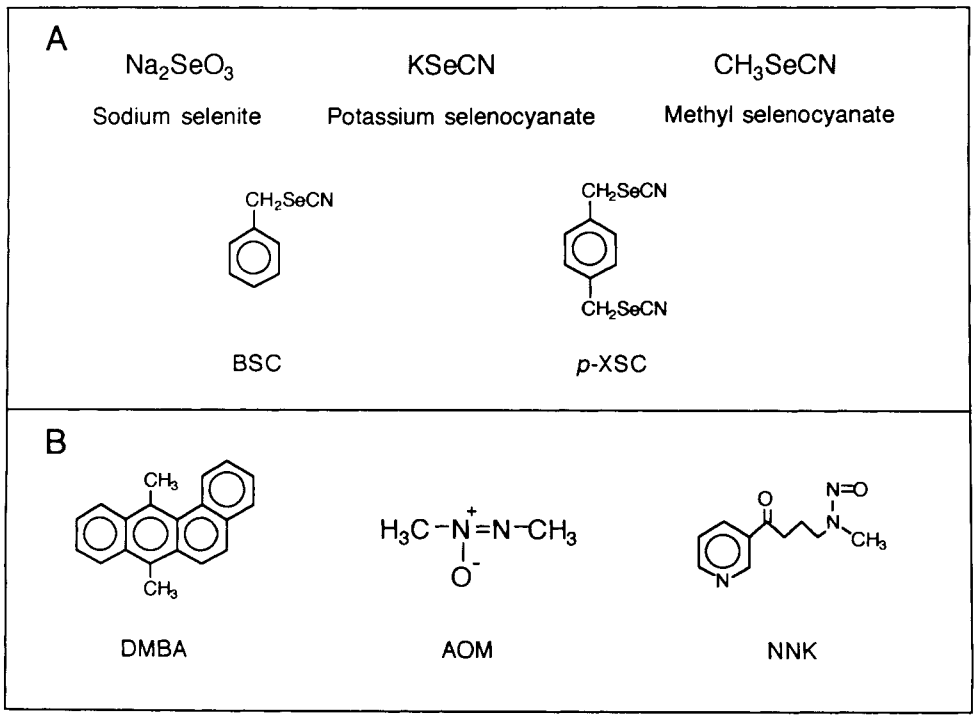

Fig. 1. Structures of chemopreventive agents [A] and carcinogens [B] used in this study.

mercaptopurine [13]. Accordingly, we substituted selenium for oxygen and/or sulfur in known inhibitors of chemical carcinogenesis. The resulting organoselenium compounds (the first generation of such agents) were examined for chemopreventive activity in various animal model systems and, in several instances, proved much more effective than their oxygen and sulfur analogs or inorganic selenite.

This report is not meant to provide a comprehensive review of the subject. Our discussion is limited to the utility of these agents in the prevention of cancer in three animal model systems: the mammary gland, the colon, and the lung. The mechanisms that may explain cancer chemoprevention as well as the future directions we believe should be pursued are discussed below.

CHEMOPREVENTION OF MAMMARY CARCINOGENESIS

7,12-Dimethylbenz(*a*)anthracene (DMBA) is the classical carcinogen used to induce mammary tumors in rats and mice, but compounds such as 2-acetylaminofluorene and *N*-methyl-*N*-nitrosourea have also been employed for this purpose [14]. We conducted studies using the DMBA-induced rat mammary tumor model system to examine the effects of organoselenium compounds on the initiation and post-initiation phases of carcinogenesis.

Benzyl selenocyanate (BSC) represents the first generation of synthetic organoselenium compounds developed in our laboratory. BSC inhibited DMBA-induced mammary tumors in rats; its sulfur analog, benzyl thiocyanate (BTC), and selenite had no effect during the initiation phase of carcinogenesis [15]. The presence of selenium in BSC is necessary for its chemopreventive activity, as is evident from comparative studies of BSC and its sulfur analog, BTC. To provide information on the possible forms of selenium responsible for the chemopreventive effect, we focused on elucidating the structures of BSC metabolites *in vivo* (Fig. 2). The results indicated the formation of benzyl selenol, benzyl seleninic acids, and dibenzyl diselenide as metabolites of BSC; in addition, evidence for methyl selenol as a metabolite of BSC was also obtained [16]. Several studies in the literature suggest that the chemopre-

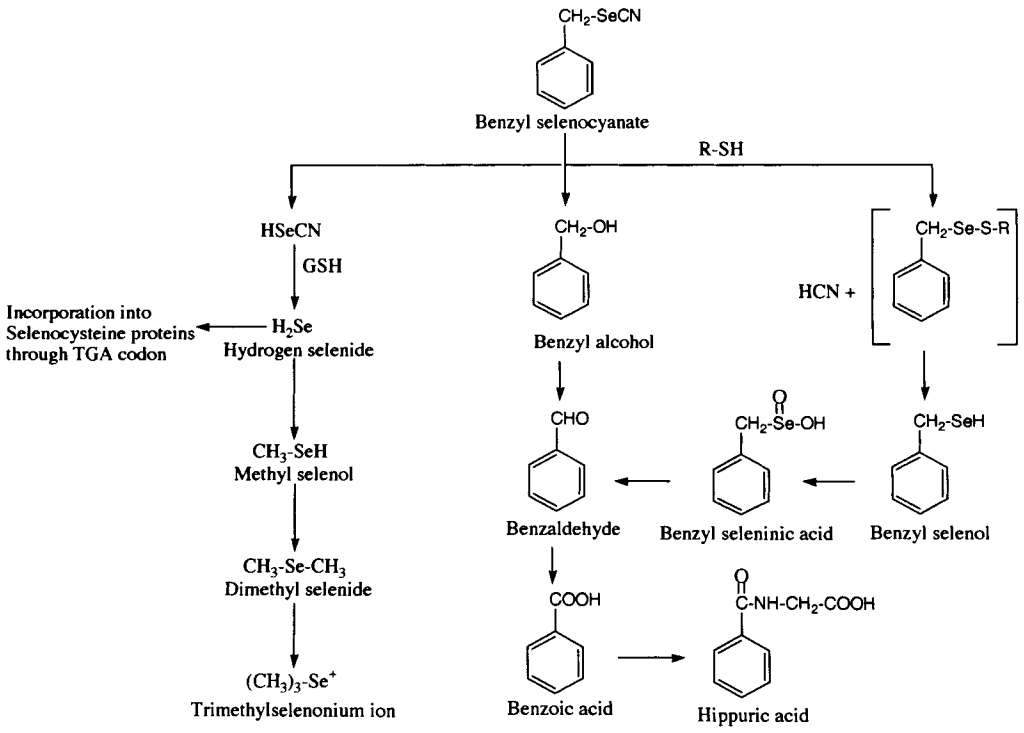

Fig. 2. Metabolism of benzyl selenocyanate.

ventive activity of selenite may be directly mediated by methyl selenol [17,18]; benzyl selenol, an analog, may be acting similarly. Further experiments are needed to obtain more evidence for the formation of benzyl selenol from BSC and to elucidate its role in chemoprevention.

Although BSC was more effective and less toxic than sodium selenite, subchronic toxicity studies in rats indicated that the body weights of rats given dietary BSC or selenite were significantly lower than those of control animals, suggesting that these agents either exerted a systemic toxic effect or induced avoidance of food [15]. Therefore, we embarked on an effort to design more effective organoselenium compounds with minimal toxic side effects. Various structural analogs of BSC were synthesized. Toxicity studies indicated that 1,4-phenylenebis(methylene)selenocyanate (*p*-XSC), representing the second generation of synthetic organoselenium compounds, was markedly less toxic than BSC [19]. We then assessed the efficacy of dietary *p*-XSC (40 ppm as selenium) in protecting against DMBA-induced mammary carcinogenesis during the initiation phase [20]. Finding that *p*-XSC inhibited DMBA-induced mammary carcinogenesis prompted us to examine the effect of dietary *p*-XSC on DMBA-DNA binding in both the liver and mammary glands under conditions identical to those in the bioassay. We found that dietary *p*-XSC inhibited total DMBA-DNA binding in the mammary glands but not in the liver [20]. The most profound effect was observed soon after DMBA administration, *i.e.*, 24–48 hr. The inhibition of total binding was attributed to a reduction in the formation of the three major adducts

TABLE I. DMBA–Induced Mammary Adenocarcinomas in Female Sprague–Dawley Rats Fed *p*–XSC and Selenite During Initiation and Post-initiation Phases[a]

Group	Experimental diets fed during[b]		% Animals with Mammary Adenocarcinomas[c]	Total Tumor Yield[d]
	Initiation	Post-initiation		
1	Control diet	Control diet	88	61
2	*p*-XSC (5 ppm)	Control diet	60[e]	41[e]
3	*p*-XSC (10 ppm)	Control diet	48[e]	30[e]
4	*p*-XSC (15 ppm)	Control diet	24[e]	12[e]
5	Selenite (3 ppm)	Control diet	68	46
6	Control diet	*p*-XSC (5 ppm)	68	47
7	Control diet	*p*-XSC (10 ppm)	56[e]	39[e]
8	Control diet	*p*-XSC (15 ppm)	48[e]	32[e]
9	Control diet	Selenite (3 ppm)	52[e]	30[e]

[a]Female Sprague-Dawley rats we maintained on the AIN-76A diet (substituting dextrose for sucrose) for the entire duration of the experiment. Mammary tumors were induced by ig administration of 10 mg DMBA at 55 days of age and rats were sacrificed between 24 and 25 weeks after DMBA treatment; [b]initiation, experimental diets were fed from two weeks before to one week after DMBA treatment; post-initiation, experimental diets were fed from one week after DMBA until the end of the experiment. *p*-XSC was added to the diet at a concentration of 5, 10, and 15 ppm as selenium; selenite was added at 3 ppm; [c]logistic regression for tumor incidence data: dose-response for groups 2–4, $p < 0.01$; dose-response for groups 6–8, $p < 0.05$; initiation *vs* post-initiation, $p = 0.08$. Polynomial regression for total tumor yield data: dose response for groups 2–4, $p < 0.01$; dose-response for groups 6–8, $p < 0.01$; initiation *vs* post-initiation, $p < 0.06$; [d]includes both palpable and non-palpable tumors; [e]$p < 0.05$ compared to the corresponding control.

derived from bay-region diolepoxides of DMBA (anti-diolepoxide:deoxyguanosine, syn-diolepoxide:deoxyadenosine and anti-diolepoxide:deoxyadenosine). Although details of the mechanism still are not clear, inhibition of DMBA-DNA binding in the target tissue provides a plausible explanation for the chemopreventive effects of *p*-XSC.

In a follow-up study, we extended these earlier observations by investigating the response to lower levels of *p*-XSC (5, 10, 15 ppm as selenium) when given either before or after DMBA administration [21]. The results clearly indicated that the chemopreventive activity was not limited to the initiation stage of carcinogenesis but was also exerted at the stage of postinitiation (Table I). In this study, the anti-carcinogenic activity of methyl selenocyanate, BSC, and *p*-XSC were also compared to that of potassium selenocyanate (KSeCN) and selenite. Cancer inhibition was ranked by calculating the ratio of maximum tolerable dose to the effective dose which produced approximately 50% inhibition in total tumor yield; this ratio is referred to as the chemopreventive index. The chemopreventive index of *p*-XSC was estimated as 4, while that of BSC, methyl selenocyanate, KSeCN, and selenite were 2.5, 2.0, 1.3, and 1.3, respectively.

To investigate the possible mechanism(s) for inhibition of the postinitiation phase of carcinogenesis by *p*-XSC, Thompson *et al.* [22] compared the effects of *p*-XSC and sodium selenite, an established chemopreventive agent shown to be a growth inhibitor, on mouse mammary carcinoma cell lines [23]. Treatment with *p*-XSC caused a 3- to 6-fold greater accumulation of selenium within cells than did treatment with equivalent amounts of selenite; also, cells were able to tolerate higher levels of selenium when it was derived from *p*-XSC. Both compounds effected a dose-dependent reduction in cell number after 24 hr of exposure and a dose-dependent increase in cell death by apoptosis. The effect of *p*-XSC on apoptosis appeared more pronounced than that of selenite. Inhibition of cell growth and induction of apoptosis may partially account for the chemopreventive properties of selenite and *p*-XSC. Tillotson *et al.* [24] compared the effects of *p*-XSC and selenite on mammary tumor cell lines derived from both humans and rats. These investigators demonstrated that *p*-XSC was capable of inhibiting thymidine kinase, while equal concentrations of selenium in the form of selenite had no effect. Collectively, the results indicate that *p*-XSC is more effective than selenite as a cancer chemopreventive agent.

CHEMOPREVENTION OF COLON CARCINOGENESIS

Experiments conducted in our laboratory have shown that the first generation organoselenium compound, BSC, but not its sulfur analog, BTC, significantly inhibited azoxymethane (AOM)-induced colon carcinogenesis when administered during the initiation or postinitiation stage of carcinogenesis [25]. In contrast, selenite inhibited colon carcinogenesis only during the postinitiation stage. It is possible that the chemopreventive effect of dietary BSC is in part mediated by inhibiting the activity of ornithine decarboxylase in the rat colon [25].

Fiala *et al.* [26] showed that BSC induced cytochrome P-450 2E1 as well as total cytochrome P-450 in rat liver. The increase in cytochrome P-450 2E1 enhances the first-pass metabolism of the carcinogen in the liver, thus decreasing delivery of activated metabolites to the target organ. BSC supplementation reduced the levels of O^6-methylguanine and 7-methylguanine in the colon but not in the liver of rats treated with AOM, in good agreement with the inhibitory action of BSC in colon carcinogenesis [25]. A more recent study was designed to investigate the chemopreventive effect of the less toxic second generation organoselenium compound synthesized in our laboratory, *p*-XSC, in AOM-induced colon carcinogenesis during the initiation and postinitiation stages in male F344 rats on a high-fat diet [27]. The rationale for the high-fat diet was to simulate the Western-style diet; also, a high-fat diet containing corn oil elevates colonic mucosal prostaglandin E_2 (PGE_2) levels compared to a low-fat diet. This study demonstrated that dietary *p*-XSC administered at 40% or 80% maximum tolerated dose levels (equivalent to 10 or 20 ppm as selenium, respectively) significantly inhibited AOM-induced colon carcinogenesis at both initiation and post-initiation stages (Table II).

Based on a mechanistic knowledge, short-term assays are routinely used in our laboratory to design less toxic and more effective organoselenium chemopreventive agents. One such assay uses rat aberrant crypt foci (ACF) formation.

TABLE II. Azoxymethane-induced Colon Carcinogenesis in Male F344 Rats Fed *p*-XSC and Selenite During Initiation and Post-initiation Phases

Group[a]	Initiation	Post-initiation	% Animals With Colon Adenocarcinomas	Mean Colon Tumor Multiplicity (Tumor/Animal ± SD)
1	Control diet	Control diet	80	1.73 ± 1.2[b]
2	*p*-XSC (10 ppm)	Control diet	63	1.00 ± 0.9
3	*p*-XSC (20 ppm)	Control diet	50[c,1]	0.80 ± 0.9[c,1]
4	Control diet	*p*-XSC (10 ppm)	40[c,2]	0.46 ± 0.62[c,2]
5	Control diet	*p*-XSC (20 ppm)	37[c,2]	0.53 ± 0.81[c,2]
6	Control diet	Control	80.6	1.5 ± 0.18
7	Selenite (4 ppm)	Control	69.4	1.3 ± 0.19
8	Control	Selenite (4 ppm)	41.7	0.72 ± 0.17[c,1]

[a]Number of animals in each group, 30–36; [b]mean ± SD; [c]significantly different from the control diet group, [1]$p < 0.05$; [2]$p < 0.01$; tumor incidences were analyzed by the χ^2 method and Fisher's exact test; the significant differences between the means were tested by Student's *t* test and analysis of variance.

Consistent with the chemopreventive activity of BSC and *p*-XSC, both agents demonstrated the capability of inhibiting ACF in rat colon following administration of AOM [28]. Structure-activity studies along this line are being conducted to optimize the design of organoselenium chemopreventive agents. Although *p*-XSC had no effect on liver cytochrome P-450, it induced UDP-glucuronyltransferase (UDPGT) in the liver. However, *p*-XSC did not alter the levels of DNA methylation in rat colon following AOM administration [29]. Such findings cannot explain the chemopreventive effect of *p*-XSC during the initiation stage of carcinogenesis; further studies are required toward this end. However, *p*-XSC inhibited PGE_2 levels and enhanced glutathione peroxidase (GSH-Px) activity in the rat colon which may in part account for the chemopreventive activity of *p*-XSC during the postinitiation stage of carcinogenesis [27]. The results of the ACF assay, DNA methylation by AOM, and colonic GSH-Px and PGE_2 may constitute the basis for utilizing these short-term assays to design better chemopreventive agents in future studies.

CHEMOPREVENTION OF LUNG CARCINOGENESIS

The magnitude of the human lung cancer problem, especially among cigarette smokers, makes it desirable to consider *p*-XSC in chemoprevention of this type of cancer. Therefore, we have set up model studies on lung cancer induction and prevention in laboratory animals. The carcinogen in this case is 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a nicotine-derived nitrosamine present in tobacco and tobacco smoke; NNK is strongly implicated in the pathogenesis of tobacco-related lung cancer in humans [30].

The well-established A/J mouse lung adenoma assay was chosen for these investigations since the lung is the major target organ for NNK-induced tumorigenesis [30]. Bioassays testing the efficacy of selenite on the inhibition of tumor induction by nitrosamines and nitrosamides are scarce. The results of these limited assays generally indicate that as a chemopreventive agent, selenite is either ineffective or extremely weak [reviewed in 3]. Selenite has not been tested in the NNK model. The experiments

TABLE III. Effects of Dietary *p*-XSC and Selenite on NNK Tumorigenesis in A/J Mouse Lung

Group	Treatment[a] (Selenium levels in ppm)	Lung tumors per mouse ± SD
1	Saline + NNK	7.6 ± 2.9
2	*p*-XCS (5) + NNK	4.1 ± 2.3[b]
3	*p*-XCS (10) = NNK	3.3 ± 2.2[b]
4	*p*-XCS (15) + NNK	1.8 ± 1.2[b]
5	Selenite (5) + NNK	8.5 ± 5.9

[a]Groups of 20 female A/J mice were fed AIN-76A diet (group 1) or AIN-76A diet containing *p*-XSC (groups 2-4) or selenite (group 5) at levels of senium indicated. Feeding started one week prior to NNK administration (ip injection of 10 μM of NNK in 0.1 ml of saline) and continued until termination of the experiment 16 weeks after carcinogen administration; [b]significantly fewer tumors than NNK alone (group 1), $p<0.0001$; tumor multiplicities were compared using Student's *t* test. Neither *p*-XSC nor selenite had an effect on tumor incidence.

in A/J mice described here provide a comparison of the efficacy of *p*-XSC and sodium selenite as inhibitors of NNK tumorigenesis [31].

The chemopreventive effect of 5, 10 and 15 ppm (as selenium) of *p*-XSC on lung tumor induction by NNK was examined in female A/J mice by administering *p*-XSC in the diet. Sodium selenite (5 ppm selenium) was given in the same manner for comparison with *p*-XSC. Mice were fed experimental diets containing the selenium compounds one week before ip injection of 10 µmol NNK in 0.1 ml saline and throughout the experiment until termination at 16 weeks after carcinogen administration. *p*-XSC significantly decreased lung tumor multiplicity from 7.6 tumors per mouse in the control group to 4.1, 3.3, and 1.8 tumors per mouse in animals given 5, 10, and 15 ppm of selenium, respectively (Table III). In contrast, 5 ppm sodium selenite had no protective effect against lung tumor induction.

In an effort to further delineate the mechanism of action of *p*-XSC inhibition of NNK-induced lung neoplasms, we examined its effect on NNK-induced DNA methylation in the lungs and liver of A/J mice. The effect of selenite was also investigated in this study. The results (unpublished) indicate that *p*-XSC (15 ppm as selenium) inhibited 7-methylguanine formation by approximately 50% after 4 hr. Dietary *p*-XSC (5 or 15 ppm as selenium) also inhibited formation of O^6-methylguanine and 7-methylguanine in the lung; however, selenite had no effect. The preliminary findings suggest that the inhibition of DNA adduct formation in the lung may, in part, account for the chemopreventive effect of *p*-XSC.

SUMMARY AND FUTURE DIRECTION

The synthetic organoselenium compounds, in contrast to selenite, can be tailored to achieve greater chemopreventive efficacy with minimal toxic side effects by structural modifications. This approach appears very promising and the results support this strategy. Similar approaches were used in cancer chemoprevention by retinoids [32]. Currently, we are working under the hypothesis that the chemical structure of the organoselenium compound (*e.g.*, *p*-XSC) could affect the chemopreventive activity *per se* and also could determine the rate of release of selenium from the parent compound into the selenium pool, thereby impacting the anti-carcinogenic efficacy, tolerance, and bioavailability of the compound. Selenium can inhibit carcinogenesis at the cellular and/or molecular level. The acquisition

of basic data for these chemopreventive agents under defined protocols of carcinogenesis and anticarcinogenesis is one area that needs intensive investigation. In addition, investigating the toxicologic properties, pharmacokinetics and mechanism of action of these organoselenium compounds is also a necessary part of the chemoprevention program. Such information is required not only for a comprehensive evaluation of the efficacy of these compounds in inhibition of cancer in laboratory animals, but is also necessary for a realistic assessment of the potential these compounds may have for human application. Further knowledge is required about the levels and chemical nature of selenium in common foods, the metabolic fate and bioavailability of these agents, as well as elucidating mechanisms and forms of selenium responsible for chemoprevention in laboratory animals. The structural and functional identification of selenium-containing proteins other than GSH-Px [33,34] will also help toward our understanding of the role of selenium compounds in cancer prevention. Thus, a database of the toxicologic and pharmacologic properties of these agents and their mechanisms of action could help translate laboratory findings to the human setting.

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REFERENCES

1. Wattenberg LW: Chemoprevention of cancer. Cancer Res 45:1–8, 1985.
2. Boone CW, Kelloff GJ, Malone WE: Identification of candidate cancer chemopreventive agents and their evaluation in animal models and human clinical trials: A review. Cancer Res 50:2–9, 1990.
3. El-Bayoumy K: The role of selenium in cancer prevention. In DeVita VT Jr, Hellman S, Rosenberg SA (eds): "Cancer Prevention." Philadelphia: J.B. Lippincott Company, 1991, pp 1–15.
4. Knekt P, Aromaa AMJ, Maatela J, Alfthan G, Aaran R-K, Hakulinen T, Peto R, Teppo L: Serum selenium and subsequent risk of cancer among Finnish men and women. J Natl Cancer Inst 82:864–868, 1990.
5. Salonen JT, Alfthan G, Huttunen JK, Puska P: Association between serum selenium and the risk of cancer. Am J Epidemiol 120:342–349, 1984.
6. van den Brandt PA, Goldbohm RA, van't Veer P, Bode P, Dorant E, Hermus RJJ, Sturmans F: A prospective cohort study on selenium status and the risk of lung cancer. Cancer Res 53:4860–4865, 1993.
7. Willet WC, Morris JS, Pressel S, Taylor JO, Polk BF, Stampher MJ, Rosner B, Schneider K, Hames CG: Prediagnostic serum selenium and risk of cancer. Lancet 16:130–134, 1983.
8. Glattre E, Thomassen Y, Thoresen SO, Haldorsen T, Lund-Larsen PG, Theodorsen L, Aaseth J: Prediagnostic serum selenium in a case-control study of thyroid cancer. Int J Epidemiol 18:45–49, 1989.
9. Virtamo J, Valkeila E, Alfthan G, Punsar S, Huttunen JK, Karvonen MJ: Serum selenium and risk of cancer: A prospective follow up of nine years. Cancer 60: 145–148, 1987.
10. Hunter DJ, Morris JS, Stampfer MJ, Colditz GA, Speizer MD, Willett WC: A prospective study of selenium status and breast cancer risk. JAMA 264:1128–1131, 1990.
11. Fan AM, Kizer KW: Selenium: Nutritional, toxicological, and clinical aspects. West J Med 153:160–167, 1990.
12. Mautner HG, Chu S-H, Jaffe JJ, Sartorelli AC: The synthesis and antineoplastic properties of selenoguanine, selenocytosine and related compounds. J Med Chem 6:36–39, 1963.
13. Mautner HG: A comparative study of 6-selenopurine and 6-mercaptopurine in the *Lactobacillus casei* and Ehrlich ascites tumor systems. Biochem Pharmacol 1:169–173, 1959.
14. Welsch C: Host factors affecting the growth of carcinogen-induced rat mammary carcinomas: A review and tribute to Charles Brenton Huggins. Cancer Res 45:3415–3443, 1985.
15. Nayini J, Sugie S, El-Bayoumy K, Cohen LA, Reddy BS: Chemoprevention of experimental mammary carcinogenesis by the synthetic organoselenium compound, benzyl selenocyanate, in rats. Carcinogenesis 10:509–512, 1989.
16. El-Bayoumy K, Upadhyaya P, Date V, Sohn O-S, Fiala ES, Reddy BS: Selenium in the chemoprevention of carcinogenesis 10. Metabolism of [^{14}C]benzyl selenocyanate in the F344 rat. Chem Res Toxicol 4:560–565, 1991.
17. Ip C, Hayes C, Budnick RM, Ganther HE: Chemical form of selenium, critical metabolites, and cancer prevention. Cancer Res 51:595–600, 1991.
18. Ganther H: Selenium metabolism and function in man and animals. In Brutter P, Schramel P (eds):

"Trace Elements—Analytical Chemistry in Medicine and Biology." Berlin: Walter de Gruyter, 1984, pp 3–24.

19. Conaway CC, Upadhyaya P, Meschter CL, Kurtzke C, Marcus LA, El-Bayoumy K: Subchronic toxicity of benzyl selenocyanate and 1,4-phenylenebis(methylene)selenocyanate in F344 rats. Fundam Appl Toxicol 19:563–574, 1992.
20. El-Bayoumy K, Chae Y-H, Upadhyaya P, Meschter C, Cohen LA, Reddy BS: Selenium in chemoprevention of carcinogenesis 11. Inhibition of 7,12-dimethylbenz(*a*)anthracene-induced tumors and DNA adduct formation in the mammary glands of female Sprague-Dawley rats by the synthetic organoselenium compound 1,4-phenylenebis(methylene)selenocyanate. Cancer Res 52:2402–2407, 1992.
21. Ip C, El-Bayoumy K, Upadhyaya P, Ganther H, Vadhanavikit S, Thompson H: Comparative effect of inorganic and organic selenocyanate derivatives in mammary cancer chemoprevention. Carcinogenesis 15:187–192, 1994.
22. Thompson HJ, Wilson A, Lu J, Singh M, Jiang C, Upadhyaya P, El-Bayoumy K, Ip C: Comparison of the effects of an organic and an inorganic form of selenium on a mammary carcinoma cell line. Carcinogenesis 15:183–186, 1994.
23. Medina D, Lane HW, Tracey CM: Selenium and mouse mammary tumorigenesis, an investigation of possible mechanisms. Cancer Res 43:2460s–2464s, 1983.
24. Tillotson JK, Upadhyaya P, Ronai Z: Inhibition of thyidine kinase in cultured mammary tumor cells by the chemopreventive organoselenium compound, 1,4-phenylenebis(methylene)selenocyanate. Carcinogenesis 15:607–610, 1994.
25. Nayini JR, Sugie S, El-Bayoumy K, Rao CV, Rigotty J, Sohn O-S, Reddy BS: Effect of dietary benzylselenocyanate on azoxymethane-induced colon carcinogenesis in male F344 rats. Nutr Cancer 15:129–139, 1991.
26. Fiala ES, Joseph C, Sohn OS, El-Bayoumy K, Reddy BS: Mechanism of benzylselenocyanate inhibition of azoxymethane-induced colon carcinogenesis in F344 rats. Cancer Res 51:2826–2830, 1991.
27. Reddy BS, Rivenson A, Kulkarni N, Upadhyaya P, El-Bayoumy K: Selenium in chemoprevention of carcinogenesis 14. Chemoprevention of colon carcinogenesis by the synthetic organoselenium compound, 1,4-phenylenebis(methylene)selenocyanate. Cancer Res 52:5635–5640, 1992.
28. Rao CV, Upadhyaya P, Sohn O-S, Simi B, El-Bayoumy K, Fiala ES, Reddy BS: Evaluation of potential chemopreventive properties of organoselenium compounds in colon carcinogenesis. Proc Am Assoc Cancer Res 35:632, 1994.
29. El-Bayoumy K, Rivenson A, Upadhyaya P, Sohn O-S, Fiala ES, Kulkarni N, Reddy BS: Chemopreventive efficacy of dietary 1,4-phenylenebis(methylene)selenocyanate (XSC) on azoxymethane-induced colon carcinogenesis in rats. Proc Am Assoc Cancer Res 33:160, 1992.
30. Hecht SS, Hoffmann D: Tobacco-specific nitrosamines: An important group of carcinogens in tobacco and tobacco smoke. Carcinogenesis 9:875–884, 1988.
31. El-Bayoumy K, Upadhyaya P, Desai DH, Amin S, Hecht SS: Inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone tumorigenicity in mouse lung by the synthetic organoselenium compound, 1,4-phenylenebis(methylene)selenocyanate. Carcinogenesis 14: 1111–1113, 1993.
32. Moon RC, Thompson HJ, Becci PJ, Grubbs CJ, Gander RJ, Newton DL, Smith JM, Phillips SL, Henderson WR, Mullen LT, Brown CC, Sporn MB: *N*-(4-Hydroxyphenyl)retinamide, a new retinoid for prevention of breast cancer in the rat. Cancer Res 39:1339–1346, 1979.
33. Sinha R, Bansal MP, Ganther H, Medina D: Significance of selenium-labeled proteins for selenium's chemopreventive functions. Carcinogenesis 14:1895–1900, 1993.
34. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG: Selenium: Biochemical role as a component of glutathione peroxidase. Science 179:588–590, 1973.