Lessons from Basic Research in Selenium and Cancer Prevention¹,²

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ABSTRACT The article reviews the progress in basic research of selenium and cancer prevention during the past decade. Special emphasis is placed on the following four major areas of discussion: 1) chemical forms of selenium and anticarcinogenic activity; 2) selenium-enriched food; 3) in vitro effects of selenite vs. monomethylated selenium; and 4) aromatic selenium compounds. It is clear that basic research has contributed new knowledge to our understanding of selenium biochemistry, anticancer efficacy and regulation of cell growth. Some of this information could be ready for incorporation into the design of a second-generation selenium trial in humans. J. Nutr. 128: 1845–1854, 1998.

KEY WORDS: • selenium biochemistry • cancer prevention • animal models • cell growth regulation

To researchers working in selenium and cancer prevention, the most exciting news in recent years is the finding by Clark et al. (1996) that supplementation of free-living people with selenized brewer’s yeast was capable of decreasing the overall cancer morbidity and mortality by nearly 50%. The study was a double-blind, randomized, placebo-controlled trial involving 1312 patients (mostly men) who were recruited initially because of a history of basal cell or squamous cell carcinoma of the skin. Individuals in the treatment arm were given 200 µg Se/d for a mean of 4.5 y (average daily intake in the U.S. is about 100 µg). After a total follow-up of 8271 person-years, selenium treatment did not significantly affect the incidence of these non-melanoma skin lesions. However, patients receiving the Se-yeast supplement showed a much lower prevalence of developing and dying from lung, colon or prostate cancer. Statistical analyses verified that the relative risk of cancer developing and dying from lung, colon or prostate cancer was reduced to 0.54 (P = 0.04), 0.37 (P = 0.002) and 0.42 (P = 0.03), respectively. Despite the fact that these are major cancers in the U.S. population, they could be considered only as secondary endpoints because the trial was originally set up to determine whether selenium would decrease the incidence of skin cancer.

A randomized, placebo-controlled intervention trial is the ultimate test to evaluate the efficacy of an anticancer agent. Before Clark’s publication, there was already persuasive evidence in the literature suggesting a cancer protective effect of selenium in humans. Geographic correlation data in different regions worldwide and in the U.S. have long noted an inverse association between selenium levels in forage crops or diet and cancer mortality rates (Clark et al. 1991, Schrauzer et al. 1977, Shamberger et al. 1976, Yu et al. 1985). Several prospective and case-control studies also confirmed that people with low blood selenium had an increased risk of cancer (Clark et al. 1984 and 1993, Salonen et al. 1984 and 1985, Willett et al. 1983). Not all selenium and cancer epidemiology investigations produced uniform results because a handful of them failed to find an association (Coates et al. 1988, Knekt et al. 1988, Menkes et al. 1986, Nomura et al. 1987, Ringstad et al. 1988). The discrepancy is not unexpected because epidemiologic designs differ from one another and these diversities are frequently difficult to reconcile. Nonetheless, the potency of selenium is perhaps best exemplified by a meta-analysis of the combined data from a number of studies comparing the significance of serum selenium, retinol, β-carotene and vitamin E in relation to cancer risk (Comstock et al. 1992). Among these micronutrients, selenium emerged as the factor with the most consistent protective effect.

In view of the renewed interest in selenium and cancer, both in the scientific and lay communities, after the publication of Clark’s project, it would be timely to examine what has been achieved in basic research during the past decade. The author has been an active participant in the field for many years. A patina of personal perspective is likely to permeate this review. This review is not intended to be all inclusive of every single paper published on the subject. Instead it will focus on four areas that may suggest the direction of our collective effort in the immediate future. In the introductory paragraph of a paper written by Howard Ganther more than 10 years ago (Ganther 1986), he stated that “it is important to keep in mind that the biological activity of selenium is an expression of selenium in a wide variety of chemical compounds, and not the element per se.” This message is just as fitting now as ever and could in fact serve as the cornerstone of this review. Incidentally, Ganther has been a long-time collaborator and has contributed in many ways to much of the work in the author’s laboratory.

¹The work from the author’s laboratory was supported by National Institutes of Health grants CA27706 and CA45164 (awarded to C.I.) and Institute Core grant CA16656.
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CHEMICAL FORMS OF SELENIUM AND ANTICARCINOGENIC ACTIVITY

One fascinating aspect of selenium biology is related to its extreme potency. Selenium, in the form of selenite or selenomethionine, functions as an essential micronutrient at levels of ~0.1 ppm (mg/kg) in the animal diet, but it becomes a toxin at levels of 8–10 ppm (Jacobs and Frost 1981). At the other extreme, selenium deficiency is customarily induced in laboratory animals by the feeding of a specially formulated diet which contains <0.01 ppm Se. It should be clarified at the outset that we will not deal with the effect of selenium deficiency on carcinogenesis. The information in this particular topic is not only sketchy but also inconsistent. For this reason, the review is limited to a discussion of the effect of selenium at levels above dietary requirement, usually in the range of 1–5 ppm Se. More than 90% of the selenium cancer chemoprevention experiments have used either sodium selenite or selenomethionine as the test reagent because they are commercially available. Both of these compounds are known to suppress carcinogenesis in many animal models (Combs 1997, El-Bayoumy 1991, Ip 1986, Medina and Morrison 1988). The effect is not organ specific, because tumor inhibition has been reported in mammary gland, liver, skin, pancreas, esophagus, colon and a few other sites. In general, there is a dose-dependent response, and selenium chemoprevention can be realized in the absence of toxicity.

On the basis of a large number of experiments that used a rat chemical-induced mammary tumor model, we showed that selenomethionine was not as active as selenite in cancer inhibition (Ip and Hayes 1989). Tissue selenium concentrations in blood, liver, kidney and skeletal muscle, on the other hand, were always higher in rats given selenomethionine compared with those given selenite. Therefore the greater total body burden of selenium in selenomethionine-treated rats did not appear to confer a better protection against tumorigenesis. The question that came to mind was whether selenium metabolism is necessary for its anticarcinogenic activity.

The above postulate was supported by additional indirect evidence from our laboratory. We found that a low methionine diet significantly reduced the protective effect of selenomethionine, even though tissue selenium was actually higher in these rats compared with those given an adequate amount of methionine (Ip 1988). When methionine is limiting, a greater percentage of selenomethionine is incorporated nonspecifically into body proteins in place of methionine (see Fig. 1) because met-tRNA cannot distinguish between methionine and selenomethionine. In other words, the anticarcinogenic activity of selenomethionine is severely compromised in a situation in which it is preferentially compartmentalized into tissue proteins instead of entering the metabolic pathway.

The schematic diagram in Figure 1 shows that methylation is a well-known fate of selenium metabolism (GANther 1986). With a high intake of selenite or selenomethionine, the levels of methylated metabolites, including methylselenol, dimethyl selenide (expired in breath) and trimethylselenonium (excreted in urine), are expected to rise. Through the support of a collaborative research program with Ganther, we conducted a series of studies that were aimed at addressing the following questions: 1) Does selenium have to flow through the intermediary inorganic hydrogen selenide pool for the cancer protective effect to be manifested? 2) Does methylation of selenium enhance or diminish its chemopreventive efficacy? 3) Is the degree of methylation important? Our strategy was to select precursor compounds that were capable of delivering selenium to specific locations along the methylation pathway.

FIGURE 1 Selenium metabolic pathway. Selenomethionine can be incorporated into proteins in place of methionine because it readily acylates Met-tRNA. Alternatively it can be converted through the transulfuration mechanism to selenocysteine, which in turn is degraded to hydrogen selenide (H₂Se) by the enzyme β-lase. In contrast, selenite is metabolized to H₂Se via selenodiglutathione and glutathione selenop-ersulfide. Hydrogen selenide is generally regarded as the precursor for supplying selenium in an active form for the synthesis of selenopro- teins. The further metabolism of H₂Se involves sequential methylation by S-adenosylmethionine to methylselenol, dimethylselenide and trimethylselenonium ion.

(Fig. 2). By this approach, we hoped to be able to pinpoint more closely the active intermediate that is involved in cancer protection (Ip and Ganther 1992). For a more detailed discussion of the biochemistry of selenium metabolism and the generation of potential chemopreventive metabolites, readers are urged to refer to a recent review by Ganther and Lawrence (1997).

Selenobetaine and Se-methylselenocysteine are good precur-sors for generating monomethylated selenium. As shown in Figure 2, selenobetaine tends to lose a methyl group first before scission of the Se-methylene carbon bond to form methylse-lenol (Foster et al. 1986a). Se-methylselenocysteine, on the other hand, is converted to methylselenol directly via a β-lase reaction (Foster et al. 1986b), and unlike selenome-thionine, it cannot be incorporated nonspecifically into pro-teins. We found that both selenobetaine and Se-methylselenocysteine were more efficacious than either selenite or selenomethionine in cancer chemoprevention in the range of 1–3 ppm Se (Ip and Ganther 1990 and 1992, Ip et al. 1991).

In contrast to the above two compounds, dimethylseleno-xide undergoes rapid reduction to dimethylselenide. It had very low chemopreventive activity even at a level of 10 ppm Se (Ip et al. 1991). After a single oral dose of dimethylselenoxide, ~90% was recovered as exhalable dimethylselenide within a 24-h period (Vadhanavikit et al. 1993). Its facile conversion to dimethylselenide, which was then rapidly eliminated via the breath, could provide a plausible explanation for the low anticancer activity.

Selenobetaine methyl ester is known to undergo breakage of the Se-methylene carbon bond to form dimethylselenide directly (Foster et al. 1986a). However, the rate of conversion to dimethylselenide might not be as fast as that with dimethylselenoxide. Interestingly, the anticarcinogenic activity of
selenobetaine methyl ester was found to be comparable to that of selenobetaine (Ip and Ganther 1990). The metabolic profile studies also provided evidence that di- and trimethylated metabolites were capable of undergoing demethylation (Vadhanavikit et al. 1993). Because of the slower metabolism of selenobetaine methyl ester to dimethylselenide, some reverse traffic of dimethylselenide demethylation might occur, thereby attaining a critical level of methylselenol in this situation. The above explanation was supported by additional data indicating that there was considerably more back conversion to the inorganic H$_2$Se pool from selenobetaine methyl ester than from dimethylselenoxide (Ip and Ganther 1992).

In summary, our studies indicated that the formation of H$_2$Se is not essential for the expression of anticarcinogenic activity. Precursor selenium compounds that are able to produce a steady stream of monomethylated metabolite are likely to have good chemopreventive activity. On the other hand, selenium compounds that are rapidly metabolized to exhalable dimethylselenide are likely to be poor candidates. The degree of methylation is also an important factor. Our results showed that the fully methylated form, trimethylselenonium, was totally ineffective (Ip and Ganther 1988), probably because it was quantitatively excreted in urine (Vadhanavikit et al. 1993). The poor tissue retention of this compound might account for its low biological activity.

In an attempt to improve the anticarcinogenic activity of the monomethylated selenium derivative, we had also examined a series of aliphatic selenocyanates with increasing length of the carbon side chain, CH$_2$-(CH$_2$)$_n$-SeCN, in which $n =$ 0, 2, 4 or 6. Selenocyanates (RSeCN) were used as the carrier of selenium because they are known to be efficiently metabolized to selenols (RSeH) and therefore represent a convenient precursor compound. Our bioassay data showed that the order of chemopreventive potency for these aliphatic selenocyanates was as follows: heptyl = pentyl > propyl > methyl (Ip et al. 1995). Thus it appeared that the longer alkyln chain homologs might be superior to methyl selenocyanate. This was a novel finding and could offer further clues to the design of more powerful anticancer selenium compounds.

Selenized yeast was the supplement given to people in Clark's study (Clark et al. 1996). Contrary to previous reports in which less sophisticated methods were used in determining that selenomethionine was the major constituent in yeast, recent analysis by a state-of-the-art technique of high performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) demonstrated that selenomethionine accounted for no more than 20% of all selenium-containing materials (Bird et al. 1997). In addition to selenomethionine, the other compounds that had been identified included selenocystine, Se-methylselenocysteine and selenoethionine (representing ~20%). On top of that, there were several unidentified peaks that combined to represent 40–50% of the total. Thus the selenized yeast actually contains a cocktail of selenium in a variety of chemical forms. Among these, we have some understanding only of selenomethionine and Se-methylselenocysteine. At this time, there are no data regarding whether these different compounds exert distinctive effects on cell biology or how they might differentially affect the multistep process of carcinogenesis. Translational research generally involves the flow of applied learning from laboratories to clinics. In selenium cancer prevention, we have an unusual scenario in which a human trial ironically magnifies the paucity of knowledge in basic science.

**RESEARCH ON SELENIUM-ENRICHED GARLIC**

The intervention trial of Clark et al. (1996) is a classic example of "targeted chemoprevention" in which a particular substance is given to high risk individuals for the purpose of reducing cancer morbidity. There is a second concept of chemoprevention that is aimed at providing cancer protective chemicals to large segments of the population that are not at an increased risk because of known exposure to carcinogens, genetic predisposition or prior diagnosis of malignancy. Because of the intrinsic requirement of this plan for a wide distribution method, an expeditious way of delivering these protective agents is through the food system. Incidentally, a driving force for general population chemoprevention can be traced to the mounting epidemiologic and experimental data that strongly suggest the beneficial effects of various plant constituents present in our diet.

It is almost impossible to increase selenium intake by eating certain types of food because most common foods have a very low selenium content (Morris and Levander 1970). In the early 1990s, Ip and Lisk started a project in which they tried to enrich garlic with selenium by fertilizing the crop with water-soluble selenite salt. The idea was stimulated by the fact that plants are known to convert inorganic selenium in soil to organic selenium compounds following the sulfur assimilatory pathway (Shrift 1973). Since garlic contains an abundance of sulfur derivatives, it might be able to accumulate high levels of selenium. Initially, our goal was to see whether the idea could be put into practice and if so, to characterize the biological activities of this Se-garlic.

By controlling the intensity and frequency of selenite fertilization, Lisk was successful in cultivating Se-garlic enriched with a low of 100 ppm to a high of 1300 ppm Se dry weight. As a point of reference, natural garlic sold in the grocery stores contains <0.05 ppm Se. After harvest and processing, the Se-garlic was usually lyophilized and milled to a powder for feeding in animal research (Ip et al. 1992). We have published a series of papers with this material. Selected findings from these studies are summarized below.

A dose-dependent cancer protective effect was expressed in the range of 1–3 ppm Se in the diet (Ip and Lisk 1994a and 1994b). Total tumor yield was consistently reduced by 50–60% with 2 ppm Se supplementation. To ascertain that the efficacy of Se-garlic in cancer protection was primarily dependent on the action of selenium, we compared the effects of two batches of garlic powder with different levels of selenium enrichment, 112 vs. 1355 ppm Se dry weight. To achieve 2...
ppm Se in the diet with these two batches of garlic powder, the amount needed was 1.8% for the 112 ppm Se-garlic vs. 0.15% for the 1355 ppm Se-garlic. In this way, we could vary the intake of garlic powder by more than 10-fold but keep the intake of total selenium constant. The results from several experiments led to the conclusion that the anticancer activity of Se-garlic was primarily accounted for by the effect of selenium, rather than the effect of garlic per se (Ip and Lisk 1995).

With the use of the rat dimethylbenz(a)anthracene (DMBA) model, we reported that supplementation of Se-garlic was capable of inhibiting both the initiation and post-initiation stages of mammary carcinogenesis (Ip and Lisk 1994b). DMBA is a procarcinogen requiring metabolic conversion to the ultimate carcinogen, DMBA-3,4-diol-1,2-epoxide, which then reacts with DNA to form adducts (Dipple et al. 1983, Liu and Milner 1992). Adduct formation is therefore the first manifestation of genotoxicity by the initiated cells. After absorption from the intestinal tract, DMBA undergoes first-pass metabolism in the liver. Although the liver is not a target site for DMBA-induced carcinogenesis, DMBA adducts are known to be present in liver DNA. After leaving the liver, some of the activated DMBA metabolites travel via the circulation to the mammary gland. Thus an analysis of DMBA adducts in both mammary cells and liver would provide confirmatory information of changes in DMBA metabolism. Our research showed that three types of adducts, anti-dG, anti-dA and syn-dA, were detected in mammary gland, whereas only the first two adducts were found in liver. Prior treatment with Se-garlic resulted in a consistent reduction of all DMBA-DNA adducts in both tissues (Ip and Lisk 1995 and 1997), suggesting that Se-garlic interfered with DMBA in causing genotoxic damage to DNA.

The decrease in DMBA adducts could be due to modulation of phase I and/or phase II xenobiotic metabolizing enzymes. Phase I enzymes are members of the cytochrome P450 system, which is responsible for converting chemical carcinogens to both electrophilic and nonelectrophilic products. The enzyme P450 1A1 is believed to play a key role in the formation of DMBA-3,4-diol-1,2-epoxide (Morrison et al. 1991). Thus a reduction in the activity of P450 1A1 would be expected to cause a decrease in adduct levels. Defenses against carcinogenic injury, on the other hand, are provided by phase II enzymes (such as glutathione-S-transferase and uridine diphosphate (UDP)-glucuronyltransferase), which are involved in the removal of metabolites through conjugation with glutathione or glucuronic acid (Talalay 1992). An increase in the activity of these phase II detoxifying enzymes could diminish the availability of DMBA metabolites in interacting with DNA.

In addition to 1A1, we also examined four other liver P450 enzymes (1A2, 2B1, 2E1 and 3A4) to determine if there might be a more general effect on the P450 family. No significant alteration was detected in any of these liver P450 enzymes in rats treated with Se-garlic at 1, 2 or 3 ppm Se (Ip and Lisk 1997). In contrast, glutathione-S-transferase and UDP-glucuronyltransferase were elevated to a maximum of 2- to 2.5-fold in liver and kidney in a dose-dependent manner (Ip and Lisk 1997). Our data therefore implied that an increased detoxification of carcinogen via the phase II conjugating enzymes might represent a mechanism of tumor suppression by Se-garlic.

The lack of an effect on P450 enzymes is actually desirable. For the development of novel approaches to cancer chemoprevention, it is generally prudent to avoid targeting the P450 enzymes because of the following considerations. A given agent may suppress a particular P450 enzyme, which is important in the activation of a certain class of carcinogens. However, the same agent may enhance other P450 enzymes that are critical in activating a different class of carcinogens. Such a double-edged sword effect is a major reason for steering away from agents that act by modulating phase I enzymes. Additionally, interference with P450 enzymes may compromise the capability of drug metabolism. This is not a trivial matter because humans frequently consume a variety of drugs to combat illnesses or diseases.

In an attempt to investigate the mechanism of tumor inhibition during the postinitiation phase, we varied the duration of Se-garlic treatment to either one of the following two protocols after carcinogen dosing: 1) a continuous feeding of Se-garlic for 5 mo until termination or 2) a 1-mo feeding of Se-garlic and a return to the control diet for the remaining 4 mo. The experiment was repeated in two mammary cancer models in which rats were given a single dose of either DMBA or methylnitrosourea (MNU). Unlike DMBA, MNU is a direct alkylating agent that does not require metabolic activation. Despite differences in their chemical reactivity, both carcinogens produce predominantly mammary tumors when given systemically to rodents. In both models, we found that short-term treatment with Se-garlic for 1 mo was just as effective in cancer prevention as the continuous 5-mo regimen (Ip et al. 1996), suggesting that Se-garlic might irreversibly suppress the clonal expansion of transformed cells in their early stage of development. Plasma and mammary tissue selenium levels essentially returned to basal values within a few weeks after withdrawal of Se-garlic supplementation. Thus the outcome of cancer protection by the short-term intervention regimen was not due to a slow turnover and thus a lingering presence of selenium in the target organ or in the circulation.

The pathobiology of chemical carcinogenesis in the rat mammary gland has been well delineated (Russo et al. 1982). There is a specific structure called the terminal end bud, which is the primary site for the induction of mammary carcinoma. Within 2–3 wk after carcinogen dosing, enlargement of the terminal end bud, characterized by a localized piling up of intraductal cells, is detectable in histological sections. These cells continue to proliferate until they fill up the duct. This type of preneoplastic lesions, known as “intraductal proliferations” or IDP, is the precursor for the eventual development of palpable carcinomas. Se-garlic could conceivably inhibit or even eliminate these IDP, thereby reducing the number of premalignant lesions that are normally present in the early stage of mammary carcinogenesis. Preliminary studies from our laboratory indicated that the total number of IDP was reduced by 50% in the Se-garlic fed rats 6 wk after MNU treatment (unpublished). This observation reinforces our belief that the IDP are likely to be the target sites of selenium chemoprevention.

Further studies also showed that Se-garlic was superior to selenomethionine in terms of its anticarcinogenic efficacy (Ip and Lisk 1996). Unlike selenomethionine, which produced large increases in tissue selenium accumulation, Se-garlic caused only modest elevations (Ip and Lisk 1996). These attributes of Se-garlic became clear when Se-methylselenocysteine was identified as the major selenium-containing constituent in Se-garlic (Cai et al. 1995). The discovery was made through a collaboration between the laboratories of Peter Uden and Eric Block. Considering the Se-methylselenocysteine research (discussed in the last section) was done before the inception of the Se-garlic project, everything came around in full circle, although the coincidence was rather fortuitous.

As a prototype “designer food” for general population che-
moprevention, Se-garlic has many desirable characteristics. Because garlic is used primarily in flavoring food, there is less danger of overconsumption. At nutritional levels of selenium intake, Se-garlic provides bioavailable selenium for the maintenance of selenoenzymes (Ip and Lisk 1993). At higher levels, it has potent anticancer activity but does not cause excessive selenium accumulation because its predominant organoselenium compound, Se-methylselenocysteine, is rapidly metabolized to di- and trimethylated excretory products (Fig. 2). It induces phase II detoxifying enzymes, thereby facilitating the endogenous removal of xenobiotics. Most interesting of all, it appears to block the development of preneoplastic lesions. This mode of action is particularly suitable for reducing cancer morbidity in sporadic cases. Because Se-methylselenocysteine cannot be incorporated nonspecifically into proteins, the amount of total selenium decays quickly from various tissues upon discontinuation of Se-garlic feeding. The lack of a persistent retention in the body might alleviate the concern of selenium in humans.

**IN VITRO EFFECTS OF SELENITE AND METHYLATED FORMS OF SELENIUM**

Although a spectrum of activities has been attributed to selenium in in vitro studies, this section will focus mainly on events that are associated with cell growth inhibition. During the 1980s, there were numerous reports showing that selenite, at concentrations in the micromole range, suppressed cell proliferation in culture and induced cytotoxicity as documented by the standard cell viability assays. This topic was reviewed previously (Ip and Medina 1987, Medina and Morrison 1988). At that time, selenite was the compound of choice because it was easily available from commercial sources. When the research was shifted to the methylated selenium compounds in the early 1990s, the laboratory of Henry Thompson began generating a body of information that supported the concept of distinctive cellular responses to specific chemical forms of selenium. The work of Thompson and co-workers resulted in a series of papers that were aimed primarily at comparing the in vitro activities of selenite with that of methylselenocyanate or Se-methylselenocysteine (Jiang et al. 1993; Kaeck et al. 1997, Lu et al. 1994, 1995b and 1996) and Wilson et al. (1992).

### TABLE 1

<table>
<thead>
<tr>
<th>Endpoints</th>
<th>Selenite</th>
<th>Methylselenocyanate or Se-methylselenocysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell morphology</td>
<td>Extensive cytoplasmic vacuolization, cell detachment</td>
<td>Normal</td>
</tr>
<tr>
<td>Membrane damage</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Cell growth inhibition</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>DNA synthesis inhibition</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Cell cycle block</td>
<td>S/G2-M</td>
<td>G1</td>
</tr>
<tr>
<td>DNA single strand breaks</td>
<td>++++</td>
<td>None</td>
</tr>
<tr>
<td>Cell death</td>
<td>Necrosis</td>
<td>Apoptosis</td>
</tr>
<tr>
<td>Gadd gene induction</td>
<td>Late</td>
<td>Early</td>
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</table>

1 The above information is based on the data published in Jiang et al. (1993), Kaeck et al. (1997), Lu et al. (1994, 1995b and 1996) and Wilson et al. (1992).
use of an asynchronized mammary epithelial cell culture model (Sinha et al. 1996), it was found that Se-methylselenocysteine caused a 57% drop in cdk2 kinase activity and a 74% decrease in cyclin E-cdk2 content (therefore compatible with a G1 arrest observed in this study as well as in the studies of Thompson), whereas selenite actually increased the cdk2 kinase activity by 47% without much appreciable change (10–20% decrease) in either of the cyclins D1, E or A bound to cdk2. The selenite results were inconsistent with a S/G2-M arrest, suggesting that the inhibition of cell growth by selenite might be associated with some nonspecific genotoxic effect unrelated to regulation of cell cycle proteins.

Thompson's studies (Table 1) and the first Sinha study (Sinha et al. 1996) of cell cycling disruption were done at a single time point in cells that were not synchronized, thus making it difficult to elucidate whether the cell cycle clock was stopped or delayed. Synchronized cells, on the other hand, are able to provide more precise information on the timing of the cell cycle clock with respect to other cellular events. With this in mind, Sinha and Medina (1997) repeated the experiments with cells that were released from growth factor deprivation by refeeding them with regular medium, a method commonly employed for synchronization. Parallel cultures were set up so

A careful examination of Lanfear's study revealed some rather curious findings in that the culture control (i.e., not treated with selenium) contained a large fraction of necrotic cells. The investigators never explained the presence of all these necrotic cells 6 h after plating when the culture should be in log growth. Upon incubating the culture with 3 μmol/L of selenodiglutathione, a small subset of apoptotic cells emerged in addition to an apparent increase in the number of necrotic cells. From the paper, it was difficult to tease out the results of percentage distribution of live cells, necrotic cells, and apoptotic cells because no quantitative data were available. Nonetheless, the appearance of apoptotic cells was unmistakable because these blue fluorescent sorted cells also exhibited the typical DNA laddering pattern on gel electrophoresis.

There was one other piece of information tucked away in the paper that was of special interest. The experiment of Lanfear was done using mouse erythroleukemia cells, which are known to carry a p53 mutated gene, suggesting that a functional p53 pathway was not essential for selenium induction of apoptosis in these cells. The dissociation between wild-type p53 and apoptosis has since been described for the effect of methylselenocyanate in a mouse MOD mammary tumor cell subline with a null p53 phenotype (Kaeck et al. 1997) and for the effect of selenomethionine in HT29 colon cancer cells, which express a mutated p53 (Redman et al. 1997). Given that mutations in p53 are among the most common pathogenetic alterations in human cancers (Greenblatt 1994), an intervention mechanism based on the induction of apoptosis could provide a strong rationale for selenium chemoprevention in the human population. Further research should be focused on testing this hypothesis in vivo and on developing appropriate biomarkers associated with the control of apoptosis.

AROMATIC SELENIUM COMPOUNDS

Karam El-Bayoumy was the first to pioneer the research of aromatic selenium compounds in cancer chemoprevention in the 1980s. His idea originated from the need to develop novel agents with a lower toxicity than that of selenite and selenomethionine. The chronology started with p-methoxybenzeneselenol (Fig. 3). In collaboration with other investigators at the American Health Foundation, El-Bayoumy reported successful tumor inhibition at different sites (liver, colon and kidney) by the feeding of 50 ppm of p-methoxybenzeneselenol (equivalent to
findings with respect to cancer chemoprevention is unclear at
whereas glutathione peroxidase was significantly increased in
sequently, there was less DNA alkylation in the colon, which was
metabolism in the liver, thus resulting in a reduced delivery of
al. (1991) found that benzylselenocyanate increased its oxidative
azoxymethane and DMBA. In the case of azoxymethane, Fiala et
P450 families are involved in the activation of benzo[a]pyrene,
carcinogens at the initiation stage is intriguing because different
specificity to selenium chemoprevention. The fact that benzylsel-
benzylthiocyanate, was not effective, suggesting that there was
before to 1 wk after carcinogen administration. The sulfur analog,
benzylthiocyanate, was not effective, suggesting that there was
inhibited DMBA-induced mammary carcinogenesis in the ini-
tromulus or phase II detoxifying enzymes
adducts (El-Bayoumy et al. 1992). After an oral gavage, the percentage dose recovered in
of cancer protection. On the other hand, it took 1 ppm Se as
XSC was highlighted in a NNK lung
carcinogenesis model. Using aberrant crypt foci as the endpoint, all three compounds expressed comparable inhibitory effects: 47% for o-XSC, 49% for m-XSC and 66% for p-XSC (Reddy et al. 1994). Although the difference in biological activity was small, the isomers were not necessarily absorbed to the same extent by the intestinal
tumor promotion phase (Ip et al. 1994a, Reddy et al. 1992),
to 10 ppm Se; the schedule generally en-
other nitrosoamines.
Attempts have also been made to compare pXSC with the
closely related structural isomers o-XSC and m-XSC (o = ortho; m = meta) in the colon carcinogenesis model. Using
with the brown rat. Despite the initial intention to develop a less toxic com-
products: triphenylselenonium, diphenylselenide and methylphe-
structurally, they differ substantially in their chemical proper-
sults. Triphenylselenonium was a very effective chemopreventive
compounds directly to a benzene
ring are very stable. There are no mammalian enzymes known
that will catalyze the transfer of the benzene ring. For this
reason, we decided to examine three phenyl selenide deriva-
tives: triphenylselenonium, diphenylselenide and methylphe-
nal selenide (Fig. 3). Although they are related to each other
structurally, they differ substantially in their chemical proper-
ties. Triphenylselenonium is positively charged and amphiphilic,
whereas diphenyl selenide and methylphenyl selenide are
uncharged and lipophilic.
Triphenylselenonium was a very effective chemopreventive
agent in the experimental mammary cancer models (Ip et al.
1994b). At a level of 30 ppm Se supplemented in the diet,
total tumor yield was suppressed by 60–70% in rats that had
been treated with a mammary carcinogen. This dose level
produced hardly any accumulation of total selenium in tissues,
even under a chronic treatment condition. Preliminary studies
indicated that it was very well tolerated by laboratory animals.
No evidence of adverse symptoms was detected at levels up to
200 ppm Se. There is thus a wide margin separating the
lung tumor multiplicity from 7.6 per mouse in the control
group to 4.1, 3.3 and 1.8 per mouse, respectively. In contrast,
selenium at 5 ppm Se had no protective effect. Consistent with
the findings of these bioassays were the observations that
pXSC decreased NNK-induced O\textsuperscript{2}-methylguanine formation in lung DNA, whereas selenium failed to produce a similar response (Prokopczyk et al. 1996). In rodents, α-hydroxylation of
NNK is a major pathway of NNK metabolism (Hecht 1994). This key reaction leads to the formation of electrophiles, which can readily methylate and pyridyloxobutylate various macromolecules. The bioactivation of NNK is catalyzed by multiple P450 enzymes including 1A1, 2A1, 2B1, 2B2 and others that have not been characterized. In view of the fact that NNK is strongly implicated in the pathogenesis of tobacco-related lung cancer in humans (Hecht and Hoffmann 1988), it is important to elucidate the biochemical mechanisms by which pXSC modulates NNK metabolism as well as that of other nitrosoamines.

With the benzyl-type selenium compound such as pXSC, some selenium is released from the parent molecule into the
inorganic selenide pool. This possibility is supported by the
evidence of nutritional bioavailability of selenium from
XSC as reported by Ip et al. (1994a). However, the rate of selenium
release cannot explain entirely the anticarcinogenic activity of
pXSC. The study of Ip et al. (1994a) showed that 10 ppm Se as
pXSC was equivalent to 3 ppm Se as selenite in the efficacy
cancer protection. On the other hand, it took 1 ppm Se as
pXSC to fully replete glutathione peroxidase in a selenium-
deficient animal as opposed to only 0.1 ppm Se as selenite. Thereby, the ratio of anticancer activity to nutritional ac-
tivity for pXSC is 10, as opposed to a ratio of 30 for selenite,
suggesting that pXSC has certain inherent activity that
is independent of the release of selenium from the parent mol-
ecule.

Compounds with selenium bonded directly to a benzene
ring are very stable. There are no mammalian enzymes known
that will catalyze the transfer of the benzene ring. For this
reason, we decided to examine three phenyl selenide deriva-
tives: triphenylselenonium, diphenylselenide and methylphe-
nal selenide (Fig. 3). Although they are related to each other
structurally, they differ substantially in their chemical proper-
ties. Triphenylselenonium is positively charged and amphiphilic,
whereas diphenyl selenide and methylphenyl selenide are
uncharged and lipophilic.

Triphenylselenonium was a very effective chemopreventive
agent in the experimental mammary cancer models (Ip et al.
1994b). At a level of 30 ppm Se supplemented in the diet,
total tumor yield was suppressed by 60–70% in rats that had
been treated with a mammary carcinogen. This dose level
produced hardly any accumulation of total selenium in tissues,
even under a chronic treatment condition. Preliminary studies
indicated that it was very well tolerated by laboratory animals.
No evidence of adverse symptoms was detected at levels up to
200 ppm Se. There is thus a wide margin separating the
chemopreventive dose range and the toxic dose range. Given the cationic and bulky nature of the molecule, the high tolerance is likely due to a poor rate of absorption via the enteral route. Fecal excretion after a single oral administration of triphenylselenonium was ~78 and 8% of the dose during d 1 and 2, respectively, suggesting that a large proportion of the gavage passed through the intestinal tract with minimal recirculation (Ip et al. 1997). Considering that so little is in fact taken up by the body, the in vivo activity of triphenylselenonium is truly fascinating.

The in vitro effect of triphenylselenonium was characterized mainly by cytostasis, i.e., a decrease in cell proliferation (due to inhibition of DNA synthesis) that was not accompanied by apoptotic cell death (Yu et al. 1995a). An agent that does not induce apoptosis will not be expected to cause deletion of transformed cells. Unless it is available continuously, the ability to protect against cancer would be lost when treatment is interrupted. This is the type of response predicted for triphenylselenonium. When triphenylselenonium was given continuously during the entire period of tumor promotion/progression (a 5-mo protocol), it was very effective in suppressing the development of tumors. However, when the treatment period was shortened to 1 mo after carcinogen dosing, there was a marked decrease in efficacy (Ip et al. 1998).

At this point, it might be worthwhile to recall the data with Se-garlic in which a 1-mo treatment schedule was just as effective as the 5-mo schedule in cancer protection. As discussed in the previous section, the monomethylated selenium is a potent inducer of apoptosis. The elimination of early transformed preneoplastic cells might explain the outcome of sustaining a lower cancer risk even if treatment is discontinued after a short period of exposure to the anticancer agent.

In contrast to the high tolerance with triphenylselenonium, a significant drop in tolerance to no more than 30 ppm Se was noted with diphenylselenide (Ip et al. 1997). At this dose level, diphenylselenide was at best only half as active as triphenylselenonium in tumor inhibition. For diphenylselenide, fecal recovery was ~6 and 30% of the dose during d 1 and 2, respectively, and ~20% of the dose was recovered in the urine on each of the 2 d. The excretion profile suggested that most of the diphenylselenide dose was absorbed and that urinary excretion was a major route of elimination for diphenylselenide once it was absorbed. Even though diphenylselenide caused a two- to threefold increase in tissue selenium, it was less active than triphenylselenonium in cancer protection. The above experiments bring home the message that small changes in the structure of selenium compounds could lead to rather surprising changes in biological activity.

The surprises continued with methylphenyl selenide. Among the three phenylselenide derivatives, it was the least tolerated. A level of 5 ppm Se of methylphenyl selenide in the diet was the maximum that would produce no decreases in the urine on each of the 2 d. The excretion profile suggested that on these hundreds of chemicals will proceed at the same pace as we cross into the 21st century.

All of the human cancer intervention studies that have been completed to date, the selenium trial is by far the most successful. The Clark study has probably attracted its share of skeptics because to put it bluntly, many may consider the results too good to be true. Therefore it needs to be repeated and it should be repeated with an improved design. During the last decade, the basic research side has contributed new knowledge of the relationship linking selenium biochemistry, anti-carcinogenic potency and regulation of cell growth. Much of this information is on the verge of being ready for incorporation into a second-generation trial. The modulation of cell cycle proteins and apoptotic proteins by selenium is an emerging area of interest. Normal cells, early transformed cells and late stage preneoplastic cells may respond differently to selenium intervention with respect to these molecular pathways.

The sooner we understand the fundamental mechanism of selenium chemoprevention, the closer we will be in finding a viable strategy in reducing cancer morbidity in the human population.

**LITERATURE CITED**


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compound, 1,4-phenylene(bis(methylene)selenocyanate. Cancer Res. 52: 5635–5640.