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DEVELOPMENT ASSOCIATION

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Selenium and The Prevention of Cancer

Part II: Mechanisms for the Carcinostatic Activity of Se Compounds

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There are two distinct areas of biological selenium research that have been conducted over the last 70 years. These very different subject areas are selenium's nutritional essentiality and toxicity. Selenium toxicity was first to be researched in the United States because of the consequences of selenium accumulation by plants from soils. These selenium-accumulating plants had such an adverse effect upon grazing cattle, sheep and horses that they impacted the livelihood OF RANCHERS in the intermountain area of the Western United States in the first half of the twentieth century. The selenium accumulator plants served as forage for open range animals and were found to be toxic when ingested¹.

Research interest into selenium toxicity waned in 1957 with the then unbelievable discovery and report that selenium was an essential nutrient for rats². Nutrition research really got under way when it was discovered soon thereafter that selenium deficiency existed in livestock populations. Diseases, which had been described as white muscle disease in sheep, mulberry heart disease in swine, exudative diathesis in chickens, and ill thrift disease conditions in other animals, were in fact manifestations of a dietary selenium deficiency. Human selenium deficiency diseases, Keshan Disease (a human heart disease) and Kaschin-Beck disease (a human rheumatoid condition) turned up in the 1980's in China and Russia. These and other diseases were all

readily prevented by the addition to diets of almost immeasurable amounts of selenium salts.

An additional research impetus into the effects of selenium came in 1973 with the discovery that the nutritional requirement for selenium fulfilled the need for an antioxidant enzyme, glutathione peroxidase³. Selenium became known as "the essential poison". Thus time and research efforts would make it clear that "the essential poison" was a part of several enzymes and proteins, found as the amino acid, selenocysteine. Within this same period of time, epidemiological evidence was also accumulating that a low dietary intake of selenium by humans may also be a measurable risk factor for cancer and heart disease. As described in Part 1, (Bulletin of the STDA, May 2001, www.stda.be/bull011.pdf) it soon became clear from many animal experiments from about 1980 forward, that supranutritional dietary supplements of selenium could prevent certain types of cancer in animals and humans.

Many selenium compounds have been shown to be effective in preventing the initiation of cancer at supranutritional levels of dietary intake in animals and humans. To understand the role of dietary selenium supplements in cancer chemoprevention an appreciation of normal dietary selenium intake and selenium supplementation need be compared. Taking animals

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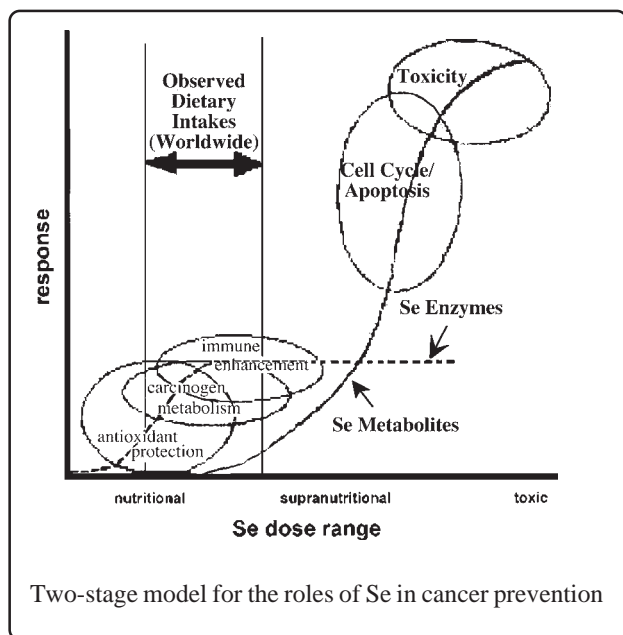


Figure 1 • Two-stage model for the roles of selenium in cancer prevention. From Combs and Grey, 1998, Reference 16. Chemoprevention as discussed below appears to take place mainly in the Cell Cycle Apoptosis area of the supranutritional dose range, Hypothesis 5.

first, most animals require between 0.10 and 0.30 mg Se/kg of diet. For disease chemoprevention in animals selenium compounds need to be supplemented to the diet in amounts of approximately 2.0-30.0 mg Se/kg of diet. This wide range in efficacy suggests that there are qualitative factors about the different selenium compounds that affect chemoprevention activity in animals. And indeed there are. Most adult humans consume 40-150 μg Se/day from their diet with an absolute minimum selenium dietary requirement of ca. 20 μg Se/day. American adults are estimated to consume in their diet without supplements, 70-120 μg Se/day. This dietary selenium is mostly L-selenomethionine from cereal grains and animal protein along with lesser amounts of L-selenocysteine from animal meats, poultry, fish and dairy products^{4,5}. Only very small amounts of other selenium compounds are to be found in the normal diet. In the limited number of human chemoprevention studies done selenium has been supplemented at about 200 μg Se/day. Thus animal studies indicate that 10-150 times the dietary amount of selenium is required for chemoprevention levels of dietary intake, dietary levels not tested in humans.

In animal studies of chemoprevention, up until the last several years, selenite had been the experimental chemopreventative agent of choice added to animal diets at 1-3 mg Se/kg of diet. Whereas chemoprevention

is obtained at these levels of dietary selenium, selenite can become toxic at just 5 mg Se/kg of diet. Because selenite is not normally found in the diet and in comparison to other selenium compounds is much more toxic, recent research has focused upon a variety of natural and manmade organic selenium compounds⁶. Most recently chemoprevention studies in animals has mostly focused attention on two dietary selenoamino acids, L-selenomethionine and L-Se-methylselenocysteine⁷. Human studies have employed mostly selenium-yeast know to contain mostly L-selenomethionine⁸. Other selenium compounds extensively studied in animals have included the manmade compounds, phenylenebis(methylene)selenocyanate (p-XSC)⁹, triphenylselenonium ion¹⁰, and most recently Se-allylselenocysteine¹¹. These efforts have all been directed to find efficacious selenium compounds for chemoprevention without associated toxicity.

Cancer and Selenium

Initiation of cancer is caused by a carcinogen, a foreign chemical, a natural metabolite, or radiation such as x-rays and UV-light and pollutants such as caused by smoking. Initiators cause genetic damage that results in mutations of deoxyribonucleic acid (DNA) that may lead to cancer. Selenite, selenomethionine, 1,4-phenylene-bis(methylene)selenocyanate, (p-XSC), other aliphatic selenocyanates, (RSeCN), selenobetaine, Se-methylselenocysteine (SeMC) as well as other selenium compounds have been shown to inhibit the induction of cancer most notably in the dimethylbenzanthracene, DMBA-induced mammary tumor animal model¹² and the dimethylhydrazine, DMH-induced colonic tumor animal model¹³, but also in the N-methyl-N-nitrosurea, NMNU-induced mammary cancer animal model¹⁴ and the azaserine, AS-induced liver cancer animal model¹⁵. How do all these many selenium compounds work to prevent cancer experimentally in animals and as shown for selenium-yeast (selenomethionine) in humans?

There are a number of hypotheses that have been postulated to account for the experimental data that selenium prevents cancer, which cautiously is often expanded to generally include all cancers. It is the purpose here, to provide an overview of the experimental evidence in support of the theories, which have been proposed to account for selenium's anti-cancer activity. Five hypotheses would seem to be possible to account for selenium's chemopreventative activity although none have been conclusively shown to be the sentinel effector. These hypotheses as adapted from Combs and Grey (Figure 1)¹⁶, and Schrauzer¹⁷ that have been postulated

to account for selenium's anti-cancer properties include; 1) selenium's antioxidant role as a component of the glutathione peroxidase enzymes, 2) selenium's enhancement of immunity, 3) selenium's effect on the metabolism of carcinogens, 4) selenium's interactions that affect protein synthesis and the cycle of cell division, and 5) the formation of anti-cancer selenium metabolites.

All of these hypotheses of cancer chemoprevention by dietary selenium remain controversial. Each is reviewed below.

1) Increases in Glutathione Peroxidases

Most research data suggest and almost all researchers agree that an adequate dietary amount of the antioxidant enzyme family of selenium containing glutathione peroxidases helps to protect against cancer, heart disease and even viral infections owing to the antioxidant role of the enzymes¹⁸. Three selenium containing enzyme classes, glutathione peroxidase(s)³ phospholipidhydroperoxide glutathione peroxidase¹⁹, and thioredoxin reductase²⁰ fulfill the known antioxidant role of dietary selenium. Since reactive oxygen species, organic hydroperoxides (ROOH) and hydrogen peroxidase (H₂O₂) are known to cause genetic damage and possibly therefore cancer²¹, selenium nutritionally acts through its enzymes, cytosolic glutathione peroxidase or membrane bound phospholipid hydroperoxide glutathione peroxidase and thioredoxin reductase to control levels of cellular hydroperoxides and the redox tone of cells that can damage proteins, cell and organelle membranes, and DNA.

The most consistent experimental effect of selenium, which reduces the incidence of experimental cancer, however, is by elevating the dietary intake of selenium in animals or humans beyond that required to synthesize all of the known selenium proteins and enzymes (Figure 1). Alternatively, selenium compounds may be injected into experimental animals gaining similar effects to dietary supplementation. In either case, normal dietary nutritional levels are exceeded and levels of synthesis of the glutathione peroxidase and other selenium proteins will be maximized. The dietary supranutritional or injected levels of selenium will reduce the risk cancer.

Selenium in the human diet consists of mostly L-selenomethionine from plant foods and L-selenomethionine and L-selenocysteine from animal foods with trace amounts of L-Se-methylselenocysteine, gamma-L-glutamyl-Se-methylselenocysteine and other selenium compounds. All of these selenium sources serve to increase glutathione peroxidase as well as other selenium proteins. While selenium and glutathione

peroxidase deficiencies can be corrected by any one of a number of dietary selenium sources and protection against cancer, heart disease and viral infections is accomplished, chemoprevention for the selenium sufficient animal or human requires pharmacological levels of selenium supplementation. Such levels of selenium supplementation do not serve to raise tissue levels of selenium enzymes and proteins further. We can say with reasonable confidence therefore, that dietary selenium supplements exert their chemopreventative effect in a manner unrelated to the saturated levels of the known selenium proteins provided by adequate dietary selenium. We must therefore look to the remaining hypotheses to account for selenium's carcinostatic attributes in selenium adequate populations.

2) Effects of Selenium on the Immune System

In 1973 the author suggested that the anticarcinogenic effects of selenium might be related to a stimulation of the immune system. This was based upon the experimental observation that dietary supplementation with selenite²² or injected selenium and/or Vitamin E enhanced the production of antibodies of mice given an immunization. Other experiments were to follow this general theme²³. Nutritional selenium supplementation was shown also to enhance the cellular immune response of the T-cell system and later on the NK cell system²⁴. Consequently selenium was shown to be selectively taken up by murine T-cells²⁵ and as reported for many animal studies supranutritional levels of selenium were then apparently stimulating all aspects of the immune system. Studies of up to eight weeks with supplemental Vitamin E and selenium in humans were shown to stimulate delayed type-hypersensitivity, enhanced immunity from the cellular immune system perhaps through the stimulation of interleukines or other associated T-cell genes. Selenium deficiency has been shown to adversely affect animals infected with viruses²⁶ and selenium deficiency is induced and is implicated in association with AIDS^{27,28}. Such comprehensive immune stimulation by selenium has been reviewed most extensively by Kiremidjian-Schumacher et al²⁹. Such effects of selenium are not yet well understood beyond the function of glutathione peroxidase and thioredoxin reductase in immune cells.

3) Effects on the Metabolism of Carcinogens

The third hypothesis is that selenium supplements interfere with the metabolism of carcinogens, chemicals both derived externally from the diet and internally from normal metabolism. Selenium may prevent cancer by

reacting directly with carcinogens to prevent their binding to DNA. This could come about by the formation of reactive selenium metabolites (as discussed in hypothesis 5 below), which could react with carcinogens making them non-carcinogenic. Selenium supplementation is known to effect the initiation of cancer by the known carcinogen DMBA³⁰ as well as by aflatoxins³¹. Such blocking of cancer initiation by selenium supplementation and direct chemical reaction of reactive selenium metabolites may be a more general phenomenon than is presently appreciated. Additionally, need for adequate dietary selenium has been shown to raise the levels of liver Phase 1 and Phase 2 xenobiotic enzymes responsible for the metabolism and detoxification of carcinogens. Several studies have found that dietary selenium supplements do not significantly raise levels of liver enzymes, the mixed-function oxidases (a hydroxylating enzyme) or cytochrome P450 that modify carcinogens, accelerate their excretion and prevent their binding to DNA^{32,33}.

4) Effects on the Cell Cycle and Protein Synthesis

Many but not all selenium compounds have dramatic effects upon the viability of cells, on the cell cycle, on protein synthesis and on DNA integrity when studied in cell culture^{34, 35}. With cells in vitro the quantitative and qualitative differences between different selenium compounds are dramatically expressed. Selenite arrests the cell cycle of cancer cells at low concentration. In doing so selenite changes the glutathione, GSH: GSSG ratio via GSH oxidation, inhibiting the G1, G2 and S-phases of cell division and protein synthesis¹⁶. Several enzymes may be adversely affected including, arginase and urease³⁶, thioredoxin reductase³⁷, and Protein kinase C³⁸. All of these enzymes contain a reactive thiol (SH) group potentially subject to oxidation by selenium compounds. In addition to affecting enzymes, selenite can cause DNA damage and cell death³⁹. Other selenium compounds can also affect the cell cycle, proteins, protein synthesis and DNA including selenodiglutathione⁴⁰, p-XSC⁴¹, methylseleninic acid⁴², selenomethionine⁴³ and Se-methylselenocysteine⁴⁴. All of these selenium compounds, as well as others, affect cells in culture and all such effects depend upon the selenium concentration. The more potent compounds that arrest cancer cell growth appear to be selenodiglutathione, selenite, selenocystine and methylseleninic acid. The less effective selenium compounds based upon dose in affecting the cell cycle in vitro appear to be the organic compounds, p-XSC, selenomethionine and Se-methylselenocysteine. Some selenium compounds like the trimethylselenonium ion are almost without any effects on the cell cycle at any

selenium concentration. The reason that some selenium compounds affect the cell cycle and protein synthesis is enzyme inhibition as noted above, and changes in the redox status of the cell, the GSH : GSSG ratio.

How these selenium compounds initiate cellular change is not widely or well understood. The answer to selenium carcinostatic activity at supranutritional intake can be explained by the formation of reactive selenium metabolites as discussed below.

5) Generation of Catalytic Selenium Metabolites

The last hypothesis is that catalytic selenium metabolites derived from the supranutritional dietary supplements of either inorganic or organic selenium compounds, natural or manmade are what contribute to selenium's carcinostatic activity and toxicity (Figure 1). The hypothesis that catalytic metabolites derived from selenium supplements account for selenium's carcinostatic activity is also the most plausible explanation for this mode of action based upon the propensity of current experimental evidence.

Past research has led to an understanding of selenium metabolism in general and the present tentative identification of the selenium metabolite likely responsible for chemoprevention is methylselenol, (CH₃SeH)^{45,47} also called the methylselenide anion, (CH₃Se⁻). The dietary consumption of inorganic selenium salts, i.e. selenite and dietary selenoamino acids leads to a reductive metabolic pathway forming hydrogen selenide, (H₂Se) which in turn is converted to the selenium amino acid found in glutathione peroxidase and other selenoproteins, L-selenocysteine. Otherwise H₂Se is methylated in three steps forming the urinary metabolite, trimethylselenonium ion (Figure 2)⁴⁶. For selenochemoprevention, a variety of metabolites in a mammary cancer model as studied by Ip and colleagues, have led to the conclusion that the active selenium metabolite for chemoprevention is methylselenol (CH₃SeH). The metabolic scheme of Figure 3⁴⁷ reveals that the most effective selenium compounds that were chemopreventive most readily forming the methylselenide anion (CH₃Se⁻), were selenobetaine and L-Se-methylselenocysteine. Additionally, in other studies, methylseleninic acid (CH₃SeOOH), when fed to animals, which easily forms the methylselenide anion directly upon reduction, also possessed chemoprevention activity⁴⁵. Likewise, the naturally occurring selenoamino acid found in garlic⁴⁸ and broccoli⁴⁹, L-Se-methylselenocysteine, when supplemented to animal diets possessed significantly better chemoprevention activity than selenite or L-selenomethionine.

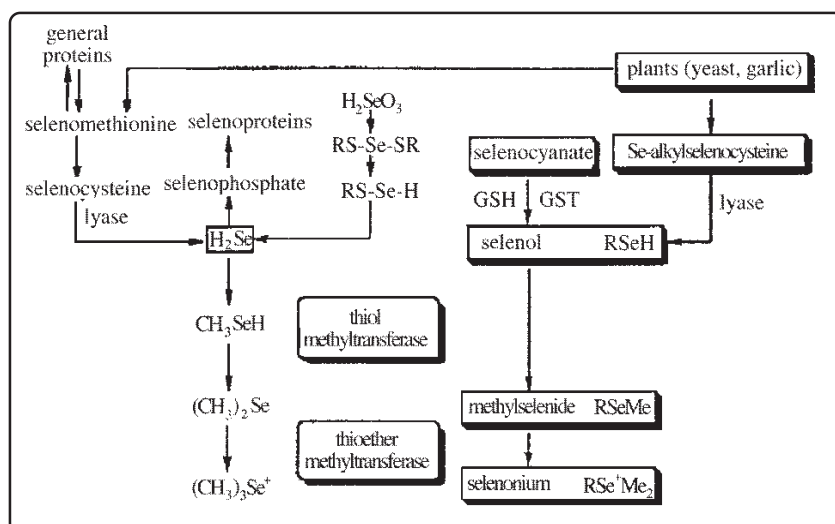


Figure 2 • Selenium metabolism, chart emphasizing reactions for generating possible chemopreventive metabolites. From Ganther and Lawrence, 1997, Reference 46. Methylation reactions play an important role in the detoxification of selenium compounds and in chemoprevention forming methylselenol (CH_3SeOH) from methylated amino acids in plants and supplements. The most potent chemopreventive natural product appears to be Se-methylselenocysteine.

Thus two selenium compounds, natural L-Se-methylselenocysteine, and manmade methylseleninic acid, appear dietarily equivalent with respect to chemoprevention in an animal model⁴⁵. These selenium compounds are not equivalent however, in a cancer cell culture assay with methylseleninic acid being the more potent in arresting cancer cell growth. Why the difference? In cell culture methylseleninic acid is rapidly reduced to the methylselenide anion by the cancer cell's glutathione, whereas L-Se-methylselenocysteine must be metabolized to form the same methylselenide anion. The cells used in this study lacked the metabolizing B-lyase enzymes and therefore L-Se-methylselenocysteine does not form the methylselenide anion. This accounts for the absence of cancer cell arrest in this study. Very recently this attribute of enzymatic activation of aliphatic selenoethers (RSeCH_3) by B-lyases or other enzymes has been demonstrated using Se-allylselenocysteine ($\text{CH}_2\text{CH}_2\text{CH}_2\text{SeCH}_2\text{NHCO}_2\text{H}$). In similar fashion this manmade selenium

compound was also inactive in a cancer cell culture assay for arresting cell growth but was active in arresting cell growth upon the addition of an enzyme, methionine-gamma-lyase⁵⁰.

The presence of these B-lyases and other enzymes naturally found in tissues has been utilized to target selenium "prodrugs" to cancer cells and to have these selenium compounds "activated" at the target cells by enzymes producing selenide anions (RSe^-)⁵¹. Thus the presence of B-lyases or similar acting enzymes in tissues holds one of the keys to understanding selenium's chemopreventive activity. Both L-selenomethionine and L-Se-methylselenocysteine are very inactive in arresting growth of cells in culture at low concentrations⁵² but are highly active in chemoprevention in vivo by likely forming methylselenols. This is due to the cellular absence of B-lyases or similar acting enzymes capable of producing the methylselenide anion within cell lines in culture but which are generated in vivo or by lyase addition in vitro.

Additional understanding of selenium's chemopreventive activity is the recent recognition that all selenium compounds that form methylselenides, (i.e. selenols) and arrest cell growth in culture could all

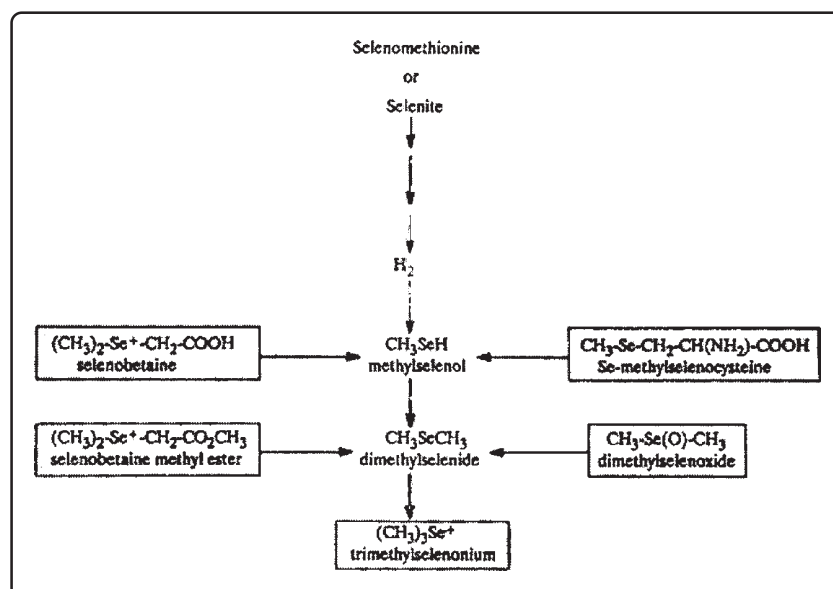


Figure 3 • Selenium metabolism, chart showing the entry of methylated products into metabolism. From Ip and Ganther, 1994, Reference 47. From the natural Se-methylselenocysteine and the synthetic metabolites (boxed selenium compounds) fed to animals the authors experimentally determined that methylselenol was (is) the likely metabolite responsible for selenium chemoprevention.

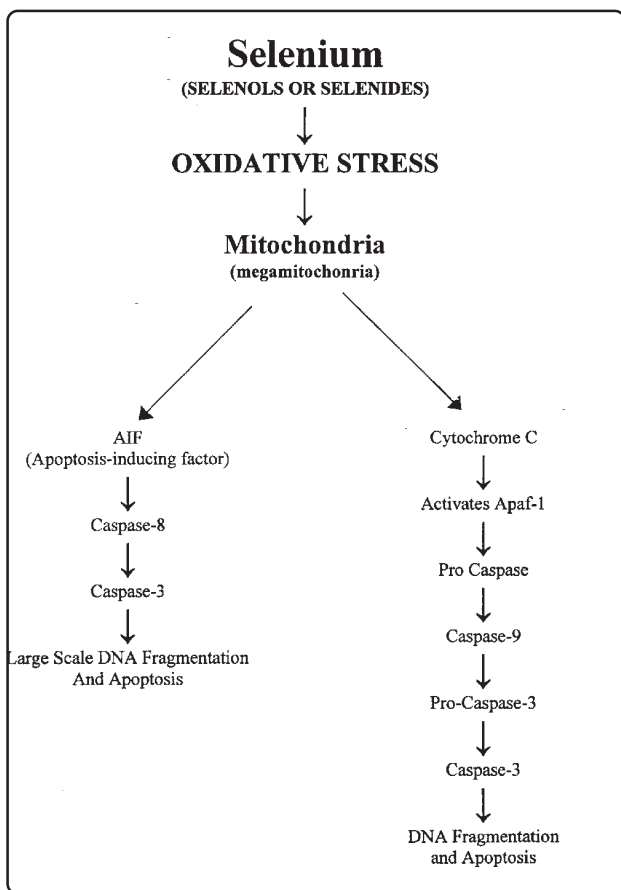


Figure 4 • Initiation of Apoptosis in Cells by Selenium. By Spallholz, 2001, unpublished. Selenium compounds, selenols or selenides induce apoptosis likely by oxidative stress causing mitochondrial swelling and release of cytochrome C. Selenium compounds that redox cycle (see Figure 5) induce apoptosis while selenium compounds that do not redox cycle do not cause apoptosis.

induce apoptosis. Apoptosis, or “programmed cell death” as it is sometimes called, is a normal event at the end of the life cycle of normal or damaged cells⁵³. Most all-normal cells die under controlled events with the exception of nerve (brain), cardiac and cancer cells. Cancer cells are normal cells that have been “transformed” by some nuclear (DNA) event and lose the normal restraints imposed by the cell’s normal life cycle. Cancer cells therefore do not die in the conventional sense but are immortalized and continue to divide ad infinitum. Apoptosis can be induced in both normal and transformed cancer cells by drugs, proteins or radiation with the controlling factor for induction of cellular apoptosis being the mitochondria of the cell^{54,55,56}.

Drugs, certain proteins and event signals outside of the cell that produce change in the integrity of the cellular mitochondria⁵⁷ induce and control apoptosis.

Mitochondria are the major site of energy transformations in cells. Disruption of the electrical potential of the mitochondrial membrane used to make energy cause it to swell and become leaky, resulting in release of cytochrome c^{58,59}. As shown in Figure 4 mitochondrial damage and release of cytochrome c initiates a cascade of cellular events that activates a series of cytotoxic proteins, the capsases that induce irreversible apoptosis.

Selenium compounds that form the methylselenide anion (selenol) at carcinostatic concentrations have recently been shown to induce cellular apoptosis. More than thirty years ago it had been shown that some selenium compounds, now known to readily form selenols, were catalytic⁶⁰ and caused what was then termed at the time, “mitochondrial swelling”⁶¹. Today we know that “mitochondrial swelling” is a precursor event to the release of cytochrome c and apoptosis⁶². At even higher concentrations selenium compounds that induce apoptosis can also cause necrosis, cellular degeneration. Often associated with apoptosis and almost always associated with necrosis is nuclear DNA fragmentation. Selenium compounds that are carcinostatic have been shown to induce nuclear DNA fragmentation as measured by the formation of electrophoretic DNA ladders, i.e. smaller fragments of DNA^{63,64}. Only one selenium compound, Se-methylselenocysteine, has been shown to date to induce apoptosis in cancer cells through activation of capsases (see Figure 4), a likely mechanism for all other selenium compounds that also induce apoptosis⁶⁴.

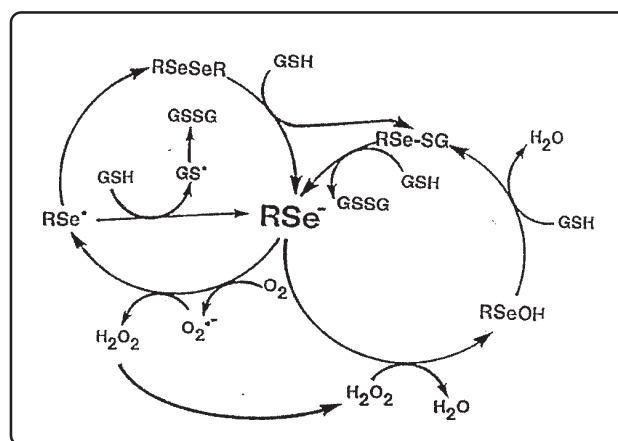


Figure 5 • Redox cycling of selenides (selenols). After Chaudière et al. Reference 75. 1992. Many selenides catalytically redox cycle, oxidizing thiols and generating superoxide and reactive oxygen species. Quantitatively sufficient selenium selenides likely induce oxidative stress in this manner and apoptosis as shown in Figure 4. Metabolism and qualitative redox cycling likely accounts for differences in the experimental chemopreventive activity of the different selenium compounds.

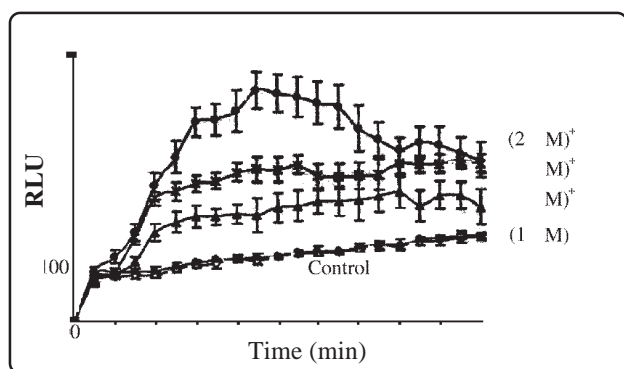


Figure 6 • Selenite-induced elevated level of superoxide formation in HepG2 cells detected by lucigenin-dependent chemiluminescent test. After Shen et al, 2000, Reference 76. Selenite at 10 μM is usually fatal to cells in culture. Shen et al followed the selenite generated release of cytochrome c, caspase-3 and DNA fragmentation

The author believes that the most compelling explanation for the associated events described above from the literature, carcinostatic activity of some but not all selenium compounds, the requirement for methylselenol formation for carcinostasis, induction of mitochondrial swelling, apoptosis and cell death lays within the ability of all selenides to generate within cells some degree of what is termed “oxidative stress”⁶⁵. In short, “oxidative stress” is quantitatively how much of an oxidizing environment is being generated over time in a cell. This can be categorized as a “lot of oxidative stress” over a short period of time or “low oxidative stress” over a longer period of time” and everything in between. The result can be the same event, induction of apoptosis only occurring within different time periods. In addition to selenides, many non-selenium compounds are known to induce “oxidative stress” and cause apoptosis, including H_2O_2 , ethanol, doxorubicin and diamide as well as many others.

The next consideration explaining the carcinostatic activity of selenium compounds is an understanding of how selenides (RSe^-) are capable of generating “oxidative stress” and apoptosis. The induction of apoptosis by selenite appears to happen because a selenide, selenopersulfide anion, is formed directly in the reaction of selenite with glutathione⁶⁶. Reduction

of methylseleninic acid by glutathione⁴², or metabolism of the selenoamino acids, L-selenomethionine or L-Semethylselenocysteine to a methylselenol⁴⁵ all produce selenides that generate a free radical, superoxide (O_2^-) as well as other reactive oxygen species^{67,68,69}. The higher the cellular selenium concentration therefore, the greater the amount of free radicals generated and the greater the “oxidative stress” produced. This basic mechanism of free radical generation and the subsequent “oxidative stress”^{70,71,72,73} as shown in Figure 5⁷⁴, can experimentally account for selenium induced apoptosis, necrosis, toxicity and most importantly, carcinostatic activity. Differences in chemoprevention between selenium compounds in literature reports are due to and can be taken into account by the different selenium compounds employed, the experimental model, the amount of selenium supplement fed, the bioavailability to the animal, the amount and form of selenium added to cells in culture, metabolism by cells, the solubility and catalytic properties of the selenides so formed and the anti-oxidative defenses of cells.

Selenite is known to induce apoptosis* in cells and the ability of selenite to induce apoptosis in cells via a free radical mechanism has just been recently published by Shen et al.^{74,75} (Figure 6). Selenite reacts with glutathione to form a highly reactive redox specie, GSSe^- , a selenopersulfide (a selenide)^{76,77} which reacts with GSH as in Figure 5 to generate superoxide. Selenite has long been known to react with GSH catalytically to reduce oxidized cytochrome c, reduce oxidized methylene blue

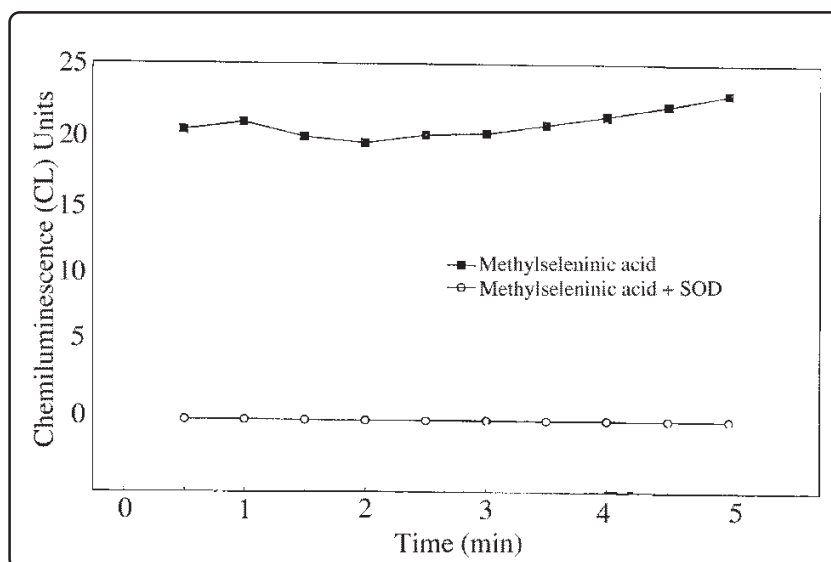


Figure 7 • Generation of superoxide by methylselenol (methylselenide) from the reduction of methylseleninic acid by glutathione in vitro. Spallholz et al, 2002⁸⁵. This reaction is continuous in the presence of glutathione and oxygen. Detection of superoxide is by lucigenin chemiluminescence with and without the addition of superoxide dismutase (SOD) which quenches the reaction.

Table 1 • A list of selenium compounds that redox cycle and do not redox cycle in vitro with glutathione. Spallholz et al, 2001⁸¹. The two natural selenoamino acids, selenomethionine and Se-methylselenocysteine do not redox cycle in vitro because they do not directly form a methylselenol. The methylselenide anion must be formed from these selenoamino acids in vivo by metabolic enzymes. The methylselenol directly formed from methylseleninic acid in vitro by glutathione is a potent redox selenium specie. Its reaction is as shown in Figure 5.

Superoxide Generated in Vitro	Superoxide not Generated in Vitro
Selenite	Elemental selenium
Selenium dioxide	Selenate
Selenocystine	Selenomethionine
Selenocystamine	Se-methylselenocysteine ¹
Diselenopropionic acid	Selenobetaine ¹
Diphenyldiselenide	Dimethylselenoxide ¹
Dibenzylselenide	Selenopyridine ⁴
1,4-Phenylenebis(methylene)selenocyanate ²	Triphenylselenonium ion ¹
6-Propylselenourcil ³	K-Selenocyanate
Dimethyldiselenide	
Methylseleninic acid ¹	

Assay conditions: 0.05M Borate buffer pH 9.2 containing glutathione (4 mg/ml) and lucigenin (20 mcg/ml) at 25 °C.

¹ Courtesy of Dr. Howard Ganther, University of Wisconsin

² Courtesy of Dr. Karam El-Bayoumy, American Health Foundation

³ Courtesy of Dr. Alvin Taurog, University of Texas Southwestern Medical Center

⁴ Courtesy of Dr. Ahmad Khalil, Yamouk University, Irbid, Jordan

and as has been mentioned, induce mitochondrial swelling, a known precursor of apoptosis. It appears therefore that mitochondria are the major target of selenium induced “oxidative stress”⁷⁸ and apoptosis⁷⁹.

In the redox assessment of many selenium compounds using chemiluminescence as a detection system for the superoxide radical, (Table 1) Spallholz et al have found that methylselenide (CH₃Se-) is also a highly reactive catalytic selenium specie in the presence of GSH that generates superoxide (Figure 7) as determined by superoxide dismutase quenching⁸⁰. Such observations support the notion that any selenide compound has potential carcinostatic activity as well as potential therapeutic applications. Without definitive proof, the inference is that differences in the chemoprevention ability of differing selenium compounds supplemented in the diet, concentration notwithstanding, resides in the enzymatic activity in vivo and within cells to generate an active selenol, the catalytic efficiency of the selenol in oxidizing GSH and other sulhydryl sensitive molecules and proteins to initially generate the superoxide radical. One thiol very sensitive to oxidation is reported to be located on the mitochondria and upon oxidation it causes release of cytochrome c and induces apoptosis⁸¹. Is this the ultimate key to selenium’s chemopreventative activity? Is it possible therefore that selenium compounds that form selenides, redox cycle producing “oxidative stress” and induce

apoptosis, perhaps via oxidation of a SINGLE sensitive mitochondrial thiol?⁸² Too simplistic? Most probably.

My final argument in support of catalytic selenides as the mechanism of selenium chemoprevention upon dietary supplementation of selenium lies within efforts to use selenides to treat disease. Chemoprevention is the use of dietary selenium supplements to prevent cancers from occurring. Nowhere to the author’s knowledge, have dietary selenium supplements been used alone in attempts to treat existing cancers. And yet this possibility exists alone or perhaps in association with conventional cancer therapies⁸³. My colleagues and I have taken an approach to develop disease therapies against cancer, bacterial and viral infections, using selenides covalently attached to site directing molecules. This is the old ‘magic bullet’ idea once held for monoclonal antibodies and recently revisited through “humanization” and genetic engineering of antibodies. We have covalently attached selenides to polyclonal and monoclonal humanized antibodies (Ab)⁸⁴ as well as peptides, steroids and even solid polymer surfaces. In the presences of thiol (GSH) in a dose dependent manner in vitro these covalently attached selenides (Ab-Se-) redox cycle and generate superoxide just as shown in Figure 5. In vitro we can use these selenide conjugates to arrest cancer cell growth in a dose dependent manner and have shown that binding is necessary for selenium to affect cell growth and viral

infectivity⁸⁵. Such experiments demonstrate that selenide redox cycling; free radical generation and subsequently formed “oxidative stress” all likely contribute to cell or viral arrest in vitro as they likely do in vivo using selenium prodrugs or dietary selenium supplements.

Summary

Selenium has been found to have three special attributes characteristic of several of the dietary trace (metals) minerals. It is dietarily essential, being incorporated into over 13 enzymes and proteins as the 21st universal amino acid, selenocysteine. It is pharmacologically active at supranutritional dietary levels and can prevent the development of various cancers (chemoprevention/carcinostatic). At higher levels of dietary intake many selenium compounds, as demonstrated in animals experimentally or in natural settings, can become toxic. All three of these attributes of selenium, essentiality, carcinostatic activity and toxicity depend upon the concentration, the chemical speciation and the catalytic activity of the selenium selenide anion (selenol). Herein lies the biological magic of selenium, catalytic activity that accounts for its essentiality in glutathione peroxidase, its pharmacology in chemoprevention and if in sufficient concentration, its toxicity. It is selenium’s redox catalytic attributes not shared to any degree by its sister element sulfur that impart all of its biological activity.

Selenium chemoprevention requires dietary supplementation with selenium beyond normal dietary intake of selenium. Supplementation continuously provides a metabolic level of catalytically active selenols. Selenols (selenides) redox cycle producing “oxidative stress” and induce apoptosis in more sensitive cancer cells. Chemoprevention likely occurs because of a differential sensitivity between cancer and normal cells to metabolically generate selenols that induce apoptosis. Differences in the effectiveness of the two leading selenoamino acid candidates for human supplementation, L-selenomethionine and L-Se-methylselenocysteine for chemoprevention will likely reside in their characteristics as substrates for enzymatic conversion to selenols and the incorporation of L-selenomethionine to body proteins.

It was once said, “that all roads lead to Rome” and presently all the collective research data leads one to the conclusion that “catalytic redox selenium metabolites are the cancer preventing agents formed by selenium metabolism in vivo.” This conclusion may even account for contributions to all of the hypotheses, 2-5, presented to explain selenium’s chemopreventative activity.

In dealing with selenium we must remember the words of Paracelsus (1393-1441), “The dosage makes either a poison or a remedy”.

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