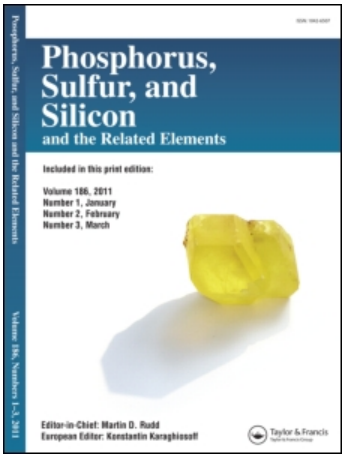

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Daniel Medina[a]; David G. Morrison[a]
[a] Department of Cell Biology, Baylor College of Medicine, Houston, Texas

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SELENIUM-MEDIATED INHIBITION OF CARCINOGENESIS

DANIEL MEDINA AND DAVID G. MORRISON
Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030

Abstract Non-toxic levels of dietary selenium exhibit potent chemopreventive activities in chemical-carcinogenesis occurring in epithelial organs. The effects of selenium are also manifested in the inhibition of cell growth in *in vitro* culture systems. Although numerous mechanisms have been proposed to explain the effects of selenium, the mechanism most consistent with the *in vivo* and *in vitro* data is a primary inhibition of DNA synthesis. Since selenium does not bind to DNA, the principal mediators are either selenoproteins or selenium-modified proteins. In the past 3 years, several new selenoproteins have been identified which provide a new avenue of research. Over the next 5 years, the research areas of selenoprotein and selenocysteine biochemistry should yield new understanding of the mechanisms of selenium function in the cell.

INTRODUCTION

The perception of the biological role of selenium and its role in carcinogenesis has changed significantly over the past 30 years. Although selenium has been recognized as an essential nutrient for normal avian and mammalian growth and function, the toxic properties of selenium have been equally recognized. In the 1940's and 1950's, selenium was viewed as a carcinogen, based on a report that rats ingesting seleniferous wheat and corn developed a high incidence of liver can-

cer.[1] In the past 15 years, the view of selenium has undergone a complete reversal and numerous experiments attest to its chemopreventive potential.[2-24] The change in perception of selenium from villain to savior has generated a great deal of interest in the mechanisms of how selenium acts as a chemopreventive agent and whether the experimental results can be translated to the human level. In this review, I will concentrate on the evidence for selenium as a chemopreventive agent and its possible mechanisms of action.

INHIBITION OF CARCINOGENESIS

There have been about 57 publications in the scientific literature which have examined the effects of selenium as a chemopreventive agent. These results are summarized in Table 1. Of the 61 experiments, 51 demonstrated an inhibitory effect of selenium supplementation on tumorigenesis, 3 showed an enhancing effect and 7 showed no effect. A cursory review of the summarized information in the table indicates that selenium inhibited chemical carcinogen-induced tumorigenesis in 9 of the 10 organ systems. It is important to note that selenium inhibited tumorigenic development in epithelial tissues which represent some of the major cancer sites in humans; namely, colon, mammary gland, liver, skin, stomach and esophagus. Additionally, the tumors were induced by a diverse set of carcinogens, including one oncogenic virus, MMTV. These data establish selenium as a chemopreventive agent with great potential for use in the human population.

It is worthwhile to note that selenium in the form of sodium selenite, also inhibits chemical and radiogenic transformation in 2 *in vitro* model systems, namely, the chemical induction of preneoplastic mammary lesions and UV-induced transformation of C3H10T1/2 cells.[25,26] Thus, model systems to study the mechanisms of selenium action are multiple and can be pursued both in whole animal and cell culture systems.

TABLE I Effect of selenium supplementation on tumorigenesis.

Organ	Species	Carcinogen [a]	Effect on tumorigenesis		
			Decrease	Increase	No effect
Liver	Rat	DAB,AAF,DEN,AFB1	8	2	0
Colon	Rat	DMH,MAM,AOM,BOP	9	0	0
Mammary Gland	Rat	DMBA,MNU,Ad-9	13	1	0
Mammary Gland	Mouse	DMBA,MMTV	11	0	3
Skin	Mouse	DMBA	3	0	0
Stomach	Mouse	BP	1	0	1
Stomach	Rat	MNNG	1	0	0
Esophagus	Rat	MBN	1	0	0
Oral Cavity	Hamster	DMBA	2	0	0
Trachea	Hamster	MNU	0	0	2
Pancreas	Hamster	BOP	1	0	1
Kidney	Rat	AOM	1	0	0

a. DAB, 3-methyl-4-diaminoazobenzene; AAF, 2-acetylaminofluorine; DEN, diethylnitrosamine; AFB1,

aflatoxin B1; DMH, 1,2-dimethylhydrazine; MAM, methylazoxymethanol acetate; AOM, ozoxymethane; BOP, bis(2-oxopropyl)-nitrosamine; DMBA, 7,12-dimethylbenzanthracene; MNU, methylnitrosourea; Ad-9, adenovirus type 9; MMTV, mouse mammary tumor virus; BP, benzo( )pyrene; MNNG, N-methyl-N'-nitro-N-nitrosoquanidine; MBN, methylbenzyl-nitrosamine.

Generalizations Concerning Selenium-Mediated Inhibition of Tumorigenesis

The results from the experiments summarized in Table 1 have yielded several general conclusions regarding the selenium-mediated inhibition of tumorigenesis. Unless stated otherwise, the form of selenium used in these experiments was sodium selenite. The inhibition of tumorigenesis occurs at non-toxic levels of selenium. In the mouse mammary tumor system, selenium at levels of 0.5 ppm inhibits tumorigenesis by 50%[2]. Since the rodent requirement for selenium is 0.1 ppm, the inhibitory levels start at 5x the nutrient requirement of selenium. Most experiments have utilized selenium at 2.0-4.0 ppm whereas minimal toxicity in rats (loss of weight gain) starts at 5.0 ppm. In humans, the minimum essential requirement appears to be approximately 30 ug/day and toxicity of chronic exposure to selenium occurs between 750 and 1000 ug/day.

Organic forms of selenium are effective inhibitors of tumorigenesis. Natural forms such as seleno-methionine[9,12] and synthetic organoselenium compounds such as p-methoxybenzeneselenol and benzylseleno-cyanate[7,18] inhibit mammary, colon and stomach

tumorigenesis. The synthetic organic forms can be used at higher dose levels.

As with many processes, the inhibitory effect of selenium can be modified by the dose of carcinogen, the type of diet, the level of fat in the diet and the genetics of the recipient host.[2,13] Selenium inhibits both initiation and post-initiation phases of carcinogenesis. In the one system where this has been systematically examined,[2,10] it appears that selenium was more effective during the post-initiation phases of carcinogenesis. However, it is also important to note that selenium must be given continuously for maximum inhibition of post-initiation phases of carcinogenesis. Upon removing animals from a selenium-supplemented diet, tumors start to reappear at approximately the same rate as in control animals. This observation is very important since it suggests that selenium suppresses the expression of transformed cells but does not eliminate transformed cells. The reversibility of selenium inhibition of cell growth has also been seen in cell culture.[2,27,28]

The chemopreventive levels of selenium used in the majority of experiments approach its toxic levels (3 vs 5 ppm in the rat). Since selenium has to be administered for a prolonged time to be effective, the proximity of the chemopreventive and toxic levels has generated a feeling of wariness about the feasibility of selenium as a chemopreventive agent. However, the demonstration that selenium synergizes with other agents to inhibit tumorigenesis suggests that low doses of selenium can be administered chronically and still be effective chemopreventive agents. In the colon system, low doses of selenium with retinoic acid and/or

B-sitoserol effectively inhibited tumorigenesis.[29] In the skin, selenium synergizes with vitamin E and/or glutathione.[15] The interaction of selenium with other agents has been systematically investigated in the rat mammary gland by Ip and his colleagues.[11,12] As summarized in the following table, selenite and vitamin E, but not selenomethionine and vitamin E, exhibited a marked synergistic effect on mammary tumorigenesis and reduced the tumor burden by 62%. In contrast, Vitamin C abrogated the chemopreventive effect of selenite but not that of selenomethionine. These results suggest a rationale for the use of low doses of selenium but also illustrate the complex interactions of selenium with other biological agents.

TABLE II Interactions of vitamins and selenium.

Agent 1	Agent 2	Effect
Selenite	-	Decrease
-	Vitamin E	No Effect
Selenite	Vitamin E	Greater Decrease (Synergism)
-	Vitamin C	No Effect
Selenite	Vitamin C	Blocks Decrease (Antagonism)
Selenomethionine	-	Decrease
Selenomethionine	Vitamin E	Decrease (No Synergism)
Selenomethionine	Vitamin C	Decrease (No Synergism/Antagonism)

Selenium is an effective chemopreventive agent. The answer to the corollary question, "Is selenium an effective chemotherapeutic agent," is laden with lack of data and uncertainties. It is well-established that selenium can significantly inhibit the *in vivo* growth of Ehrlich ascites tumor cells, L1210 leukemia cells and canine and human mammary tumor cells.[2,30] The efficacy of selenium-mediated inhibition was dependent upon the chemical form of selenium (selenite > selenate; selenite > selenocysteine > seleno-methionine), the dose of selenite and on the route of administration (i.p. > per os). In contrast, several workers [2] have demonstrated the lack of a significant inhibitory effect of selenium on the growth rate of primary mammary tumors in the mouse and the rat. In the mouse mammary gland, selenium seems to be most effective during the early stages of mammary transformation; i.e. the induction and expression of mammary preneoplasias. A reconciliation of these opposing data is difficult, however, not impossible. Tumor cell lines which have been passaged *in vivo* or *in vitro* many times represent highly selected cell populations. Thus, their response to any agent may not accurately reflect the response of a heterogenous cell population which is typical of primary tumors in *situ* or tumors in early transplant generations. The inhibitory effects of selenium on tumor cell growth in established cell lines are useful to understand possible mechanisms of intracellular selenium action, but are not appropriate to establish chemotherapeutic efficacy.

Possible Mechanisms of Selenium Action

Numerous chemical carcinogens are mutagens in the Ames Salmonella mutagenesis assay. Since selenium inhibits direct and indirect-acting carcinogens, several investigators have examined the effect of selenium on carcinogen-induced mutagenesis[2]. In the experiments where the cytotoxicity of selenium has been controlled, selenium inhibits mutagenesis induced by malonaldehyde, B-propiolactone, MNNG and AAF as well as spontaneous mutagenesis. The mechanism for this effect is unclear but should be distinguished from those conditions where selenium causes DNA damage.[2,31] In the latter case, the deleterious cytogenetic and mutagenic damage occur with high doses of selenium ($\geq 10^{-5}$M). Under such conditions, selenium has a cytotoxic effect. Thus, in any consideration about the mechanism of selenium action, both concentration and form of selenium need to be evaluated. The failure to recognize the nonspecific effects of high doses of selenium on cell function has generated many misleading conclusions in the literature.

TABLE III Suggested mechanisms of selenium action.

1.	Alteration of carcinogen metabolism
2.	Alteration of carcinogen-DNA interactions
3.	Maintenance of GSH-Px activity and/or inhibition of lipid peroxidation activity
4.	Maintain intracellular glutathione levels
5.	Indirect or direct inhibition of DNA synthesis

Since selenium inhibits both the initiation phase of carcinogenesis and carcinogen-induced mutagenesis, it was of interest to determine if selenium altered carcinogen metabolism or carcinogen-DNA interactions. Although only a few experiments have been performed, the results suggest that selenium has no consistent effect on the metabolism and binding to DNA of carcinogens such as DMH and DMBA.[2,10,32] The exception appears to be AAF where selenium shifts the balance of metabolism towards detoxification pathways and decreases the binding of labeled AAF to liver DNA.[33]

The only function characterized for selenium in mammalian cells is its antioxidant function. The enzyme, GSH-Px, is a selenoenzyme which, along with vitamin E, functions to eliminate organic peroxides from the cell. Several investigators have examined the interaction of selenium, GSH-Px activity and/or lipid peroxidation during carcinogenesis. On the one hand, it is evident that the level of GSH-Px activity is not correlated with dietary selenium levels greater than 0.1 ppm nor can supplemental selenium levels overcome carcinogen-induced depression in GSH-Px levels.[3,14] Furthermore, whereas vitamin E decreases lipid peroxidation activity in mammary cells but is not a chemopreventive agent, selenium has no effect on lipid peroxidation activity. These results argue against a role for selenium functioning as an anti-oxidant. However, in the radiogenic transformation of C3H10T1/2 cells, selenium (2.5 uM) resulted in increased levels of cellular glutathione peroxidase, catalase and glutathione and in an enhanced destruction of peroxide.[26] Similarly, selenium, alone or combined with vitamin E, blocked the TPA-induced decrease of

GSH-Px in epidermal cells.[15] Thus, in some systems (epidermal and radiogenic carcinogenesis), selenium can enhance GSH-Px activity, whereas, in other systems (mammary and colon carcinogenesis), there is no effect.

A large body of evidence has implicated selenium in the regulation of DNA synthesis. Whereas selenium at nM levels facilitates DNA synthesis and cell growth, selenium at uM levels inhibits cell growth and DNA synthesis. The inhibition of DNA synthesis has been observed on established cell lines, in primary cell culture, in regenerating hepatocytes and in colonic epithelium *in situ*.[2,25,30,32,34-37] In mammary cells grown in monolayer cell culture, the decreased cell growth is reflected by a decreased cell number, decreased uptake of ^{3}H-thymidine into DNA, decreased nuclear labeling index and a decreased rate of DNA synthesis. Flow cytofluorometry analysis of selenium-treated mammary cells demonstrated that the cells appeared to be blocked in the G_2-S phases of the cell cycle. The inhibition of cell growth and DNA synthesis has been the most consistent observation regarding its possible modes of action. Inhibition of DNA synthesis occurs prior to inhibition of general protein synthesis. However, in one experiment, inhibition of cell growth was accompanied by inhibition of protein synthesis under conditions where DNA and RNA synthesis was not altered.[38,39] The inhibition of protein synthesis was correlated with inhibition of elongation factor 2[39,40] but not eukaryotic initiation factor 2.[41]

The question of how selenium might inhibit DNA synthesis is unknown. Selenium-induced inhibition of RNA and DNA polymerases,[42] alterations in protein phosphorylation[42] and cellular glutathione[44,45] as well

as hypomethylation of DNA[46] have been reported recently. The significance of these observations with respect to inhibition of cell growth has not been well defined, although these pathways represent feasible mechanisms of selenium action. It is known that very little selenium gets into the nucleus and binds DNA, thus a direct interaction of selenium and DNA has seemingly been ruled out. On the contrary, the majority of selenium is localized in small molecular weight TCA-soluble constituents and about 10% in TCA-insoluble proteins. The characteristics and cellular localization of these proteins are beginning to be investigated. By 2-dimensional gel electrophoresis[47] and column chromatography,[48] there are 9-11 proteins which covalently bind selenium. The polyacrylamide gel electrophoresis separation of these proteins under extensive denaturing conditions has ruled out tri-sulfide formation. Rather, it appears the majority of the selenium is incorporated as selenocysteine. Under conditions where selenium inhibits cell growth, 4 major proteins and several minor proteins can be localized in the cell. Table 4 shows the cellular localization of the selenoproteins in the mammary cell line MOD. Although mitochondria accumulate a significant amount of selenium, compared to other fractions less is bound to proteins. The mitochondria are functionally normal with respect to phosphorylating respiration, P/O ratio, respiratory control index and specific mitochondrial enzyme activity. A small molecular weight protein (16K) is located primarily in the nucleus whereas the other major selenoproteins are located in the cytosol (58K) and microsomal fraction (26K,23K). The function of these proteins other than GSH-Px (26K) are unknown

and one of the immediate challenges is to purify these proteins. This aim is currently being investigated by at least 3 laboratories.

TABLE IV Intracellular compartmentalization of ^{75}Se-labeled proteins in the mammary epithelial cell line MOD.

Fraction	% ^{75}Se as protein	Selenoprotein (M.W.)[a]
Nuclear pellet	5.53	16K, 14K, 11K
Mitochondrial pellet	4.41	23K, 17K, 12K
Microsomal pellet	7.95	51K, 26K, 23K
Cytosolic supernatant	6.27	58K, 51K

a) Underlined selenoprotein is made before inhibition of DNA synthesis.

Experiments on the structure of the GSH-Px gene have already yielded unexpected and exciting results. The GSH-Px mRNA is UGA, which is normally a stop codon.[49] Since selenocysteine is in the middle of the protein, it is of fundamental interest to understand how and under what conditions a stop codon is read through by tRNAs. Opal suppressor tRNAs have been identified in eukaryotes which allow the insertion of a phosphoserine into the polypeptide chain thereby suppressing the termination of translation.[50] Mitzutani *et al.*[51] have isolated a kinase specific for seryl-tRNAs. Finally, Sunde and Evenson[52] have reported that the serine carbon skeleton comprises the carbon skeleton of the selenocysteine found in GSH-Px.

In this model where selenium is added at a cotranslational step, the enzymes responsible for adding selenium to a phosphoserine residue would be located in the cytosol.

How does the cell choose which UGA codon to read through via insertion of a phosphoserine or injection of a selenocysteine? Jacks et al.[53] proposed that secondary mRNA structure provides the appropriate signals which specify readthrough of stop codons in MMTV genes. Secondary structures, i.e. hairpin loops, also exist in the GSH-Px genes. How the cell chooses UGA codons in different hairpin loops to modify with selenocysteine rather than just phosphoserine is not known. It is possible that one of the 2 known opal suppressor tRNAs recognizes the context specificity between these hairpin loops. Context specific suppressor tRNAs have been demonstrated experimentally in several systems.[54,55]

In summary, there exists a great deal of data which documents selenium as a potent chemopreventive agent. There are several intervention trials currently being carried out around the world utilizing various forms of selenium. The biggest challenge is to understand how selenium acts as a chemopreventive agent. The pursuit of this question is pushing selenium into the sphere of immunological and molecular biology techniques. The exciting and surprising observations that the selenocysteine residues in the mammalian enzyme, GSH-Px and the bacterial enzyme, formate dehydrogenase, are encoded by UGA, which is a stop codon in other proteins, has opened up new avenues of research. It is likely that in the next 5 years, research areas on cancer chemoprevention, seleno-

proteins and selenocysteine biochemistry will converge and yield new understanding in the mechanisms of selenium function in the cell.

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