ELSEVIER

Mutation Research 475 (2001) 123–139

MR Fundamental and Molecular Mechanisms of Mutagenesis

www.elsevier.com/locate/molmut
Community address: www.elsevier.com/locate/mutres

Review

The protective role of selenium on genetic damage and on cancer

Karam El-Bayoumy*

Division of Cancer Etiology and Prevention, American Health Foundation, 1 Dana Road, Valhalla, NY 10595, USA

Received 21 March 2000; received in revised form 8 September 2000; accepted 26 September 2000

Abstract

Collectively, results from epidemiologic studies, laboratory bioassays, and human clinical intervention trials clearly support a protective role of selenium against cancer development. Several hypotheses have been proposed to explain these observations. Increased genomic instability, either inherent or induced by exogenous agents (mutagens or carcinogens), has been considered as a primary event leading to neoplastic transformation. This report deals specifically with the evidence for a role of selenium in the inhibition of carcinogen-induced covalent DNA adduct formation and retardation of oxidative damage to DNA, lipids and proteins, and for modulating cellular and molecular events that are critical in cell growth inhibition and in the multi-step carcinogenesis process. At present, the bulk of our knowledge on the role of selenium on genetic stability is based primarily on animal data and from studies conducted in in vitro systems. Studies performed in vitro showed that the dose and form of selenium compounds are critical factors with regard to cellular responses. Inorganic (at doses up to 10 μM) and organic selenium compounds (at doses equal to or greater than 10 μM) elicit distinctly different cellular responses. The recommended daily allowance (RDA) is 50–70 μg Se per day for healthy adults; with 40 μg Se as minimum requirement. Less than 11 μg Se will definitely put people at risk of deficiency that would be expected to cause genetic damage. Daily doses of 100–200 μg Se inhibited genetic damage and cancer development in humans. About 400 μg Se per day is considered an upper limit. Clearly, doses above the RDA are needed to inhibit genetic damage and cancer. However, it has been hypothesized that the intake of excessive doses of selenium may cause oxidative damage, leading to genomic instability. The use of a cocktail consisting of selenium, and other vitamins and minerals appears to be a promising approach to inhibit genetic damage and the development of cancer. It is the author's recommendation that development of mechanism-based hypotheses that can be tested in pilot studies in different populations prior to a large-scale clinical trial in humans, is of paramount importance in order to better understand the role of selenium on genetic stability and cancer. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Selenium; Cancer; Oxidative damage; Cell proliferation; Apoptosis; Micronuclei; Chromosomal damage

1. Introduction

In the US, nearly two-thirds of cancer deaths can be linked to dietary factors and tobacco use [1–6]. However, diet also provides numerous vitamins and trace minerals that are essential for normal metabolism [7]; the essential trace minerals include selenium [8]. Many foods (grain products, seafood, meat and poultry) are major sources of selenium [9]. Seafood accounts for approximately 30% of the dietary selenium intake. The structural identities of the selenium compounds in food remain largely unknown. Bioavailability differs for inorganic and organic selenium compounds [10,11]; known structures of some representative inorganic and organoselenium compounds

* Tel.: +1-914-789-7176; fax: +1-914-592-6317.
E-mail address: kelbayoumy@aol.com (K. El-Bayoumy).

0027-5107/01/$ – see front matter © 2001 Elsevier Science B.V. All rights reserved.
PII: S0027-5107(01)00075-6

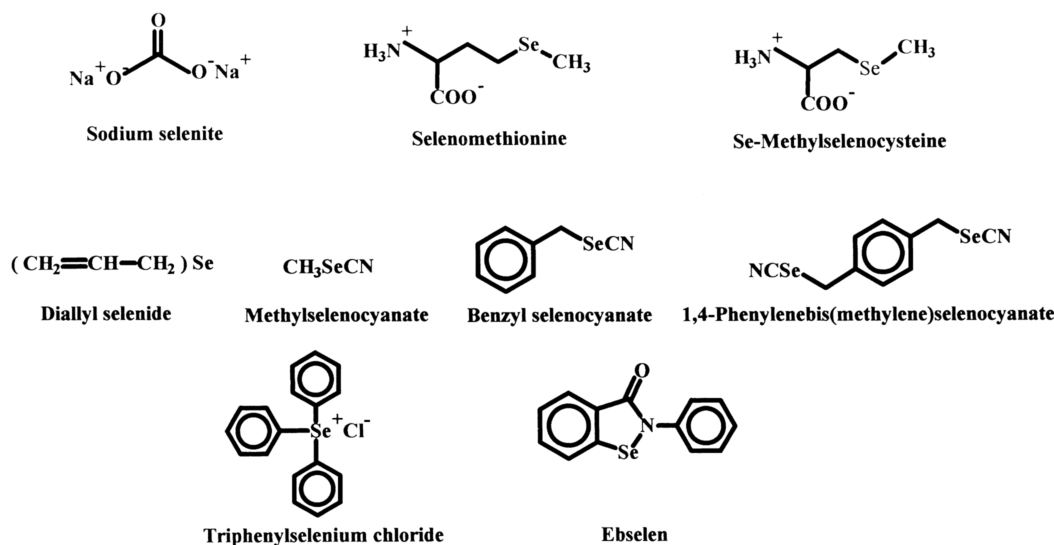

Fig. 1. Structures of representative compounds described in this review.

are shown in Fig. 1. Studies suggest that the formulation of the selenium-containing compound and not the element, per se, is critical for biological activities [12]. Therefore, it is essential to determine which structural requirements govern and which provide optimal biological activity of selenium compounds.

As a naturally-occurring element, selenium ranks 17th. Its geographic distribution varies from high concentration in the soil in certain regions of China, the former USSR, Venezuela, and the US to rather low levels in New Zealand and Finland. Surveys of dietary intake among residents in the US have shown that, according to the recommended daily allowance (RDA), a sizable percentage of the population is deficient in many micronutrients but not in selenium [13–16]. However, selenium intakes in most parts of Europe are considerably lower than in the US [17]. Selenium deficiency would be expected to induce genetic damage as a result of causing excessive oxidative stress. Selenium deficiency has been implicated as playing a role in the development of many diseases, including cancer, cardiovascular and immune disorders [17]. On the other hand, the intake of an excess of selenium may result in oxidative damage leading to genomic instability [18]. A review of the literature as presented in this report, makes it clear, however, that levels of selenium above the RDA are required for the inhibition of genetic damage and cancer in both rodents and in humans [17,19–22].

1.1. The protective role of selenium in cancer prevention

1.1.1. Epidemiological studies

The history of biomedical research on selenium, which was discovered in 1817 by the Swedish chemist Berzelius, is intriguing. Early reports identified selenium as a highly toxic element. Between 1951 and 1957, the pioneering work of Schwartz and Foltz demonstrated that selenium is an essential nutrient [23]. Further support of the benefits of selenium came after the discovery by Rotruck in 1973 of its essential role in the formation of glutathione peroxidase, an enzyme that protects against oxidative injury [24]. This nutritionally essential trace element was first associated with cancer risk approximately 30 years ago [25]. Epidemiological studies have suggested that an increased risk for certain human diseases, including cancer, is related to insufficient intake of selenium; however, there remains some inconsistency [21]. In a cohort study, men in the highest selenium quintile of intake had only one-half the odds ratio of prostate cancer of men in the lowest quintile [26]. In a nested case-control study on ovarian cancer, serum

selenium was associated with decreased risk [27]. The seleno-enzyme, iodothyronine deiodinase, is responsible for the synthesis of triiodothyronine (T_3) [17]. A strong inverse relationship was observed between T_3 levels and cancer between the highest and lowest tertiles of intake in a study of postmenopausal breast cancer patients [28]; levels of selenium in toe nails were positively associated with T_3 levels in both cases and controls. In a study of selenium intake and colorectal cancer, that adjusted for possible confounders, the individuals in the lowest quartile of plasma selenium had four times the risk of colorectal adenomas compared to those in the highest quartile [29]. Selenium and glutathione peroxidase levels were found to be lowered in patients with carcinoma of the uterine cervix [30]. In a study in China, cervical cancer mortality was inversely related to several factors, including serum selenium levels [31].

1.1.2. Clinical intervention studies

The use of selenium in human clinical trials is limited thus far (Table 1). These intervention trials have been conducted in China, India, Italy, and the US with selenium, in the form of selenium-enriched yeast, selenite, or selenate [32–39]. In certain trials, it was difficult to tease out the form of selenium that was given. Populations having different risk factors were recruited for these trials. Some of the studies performed in China suffered from methodologic problems such as lack of quality controls [32,33]. The Linxian (China) cancer prevention trials have shown that giving a combination of selenium, β-carotene, and α-tocopherol resulted in significantly fewer cases and a lower mortality from stomach cancer than were observed in the placebo groups [34]. When selenium was given in combination with another 25 vitamins and minerals, it had no effect on the development of esophageal cancer [35]. In a study conducted in India, selenium was given in combination with Vitamins A, C, and E, as well as zinc [36]. Here, the results clearly showed a protective effect of this cocktail against the development of oral lesions in subjects who practice reverse smoking. In a double-blind randomized trial in Italy, inhibition of adenoma in the large bowel by selenium has been demonstrated [37]. One of the most exciting clinical trials in the US supported a protective effect of selenium-enriched yeast against cancer of the prostate, colon, and lung [38,39]. The outcome of Clark's trial [38,39] stimulated the initiation of two new clinical intervention trials in three European countries (PRECISE) and in the US (SELECT) [17].

1.1.3. Formulation of hypotheses

Collectively, epidemiologic evidence, laboratory bioassays and human clinical intervention trials support a protective role of selenium against development of cancer. Several hypotheses, outlined below, have been proposed to explain the protection against carcinogenesis by selenium supplementation. Genetic damage, leading to the accumulation of specific mutations, is a prerequisite for the cell's transformation from a normal into a malignant phenotype. While there are several types of genomic instability, this report specifically addresses the hypotheses and provides the evidence that selenium supplementation can inhibit carcinogen-induced covalent DNA adduct formation and can retard oxidative damage as a result of chemical and physical insult to DNA, lipids, and proteins. Different forms and levels of selenium compounds are critical factors with regard to cellular responses. This review summarizes knowledge on the protective effect of selenium compounds, individually and in combination with other vitamins and minerals as it relates to cellular and molecular targets that are critical in the multi-step process of carcinogenesis. Finally, a recommendation is made to emphasize future research needs in this area.

1.2. The protective role of selenium on carcinogen-induced DNA adduct formation

1.2.1. Breast tissue

It is commonly accepted that carcinogen-induced genetic damage via the formation of covalent DNA adduct is necessary, but not sufficient, for the initiation of carcinogenesis. Therefore, several studies were conducted in vitro in rodents to examine the effects of various levels and forms of selenium on carcinogen DNA adduct formation [40–56] (Table 2). Though not a natural product but a reliable tumor initiator, the synthetic polynuclear aromatic hydrocarbon 7,12-dimethylbenz(*a*)anthracene (DMBA) has been used extensively in model assays for mammary carcinogenesis [57,58]. Both the liver and mammary tissues are capable of metabolizing DMBA to reactive bay-region diol epoxides which are involved in

Table 1
Clinical trials in the US and abroad employing selenium alone or in combination with other minerals and vitamins

Study no.	Level and form of selenium[a]	Country[b]	Population	Type of cancer (outcome)[c]	References
1	200 μg Se per day for 2 years as selenium-enriched yeast	China	Hepatitis surface antigen carriers	Liver cancer (I)	[32,33]
2	Table salt fortified with 15 mg/kg Se as selenite	China	Hepatitis surface antigen carriers	Liver cancer (I)	[32,33]
3	50 μg Se per day as selenium-enriched yeast	China	Double-blind, placebo-controlled; general population	Stomach cancer (I)	[34]
4	50 μg Se per day as selenate	China	Double-blind, placebo-controlled; esophageal dysplasia	Esophaegeal cancer (NE)	[35]
5	100 μg Se per day (6 months) and 50 μg Se per day (6 months)	India	Reverse smokers	Oral lesions (I)	[36]
6	200 μg Se	Italy	Patients with prior resected adenomatous polyps; randomized, double-blind	New adenomatous polyps (I)	[37]
7	200 μg Se per day as selenium-enriched yeast	US	Patients with prior skin cancer; randomized, double-blind; placebo-controlled design	Basal cell or squamous cell carcinoma (NE); lung (I); colon (I); prostate (I)	[38,39]

[a] In study no. 3, selenium-enriched yeast was used in combination with β-carotene and Vitamin E. In study no. 4, selenite was used in combination with other 25 vitamins and minerals. In study no. 5, selenium was administered in combination with Vitamin A, riboflavin, and zinc. In study no. 6, selenium was given in combination with Vitamins A, C and E, and zinc.

[b] In study nos. 1 and 2, populations were from Qideng, Shandeng province. These studies suffered from methodologic and quality control problems. In study nos. 3 and 4, subjects were selected from Linxian, Henan province. In study no. 5, selenium was administered daily for 1 year. In study no. 6, patients were selected from Genoa. In study no. 7, patients were selected primarily from the eastern US.

[c] I: inhibition; NE: no effect.

Table 2
The effect of selenium on carcinogen-induced covalent DNA adduct formation in rodents

Form of selenium	Carcinogen[a]	Sex, species, organ	Outcome[b]	References
Selenite	DMBA	Female, SD rat, mammae	I	[40–42]
Selenite	DMBA	Female, SD rat, mammae	NE	[43]
		Female, SD rat, liver	NE	
p-XSC	DMBA	Female, SD rat, mammae	I	[45,46]
		Female, SD rat, liver	NE	
o-, *m*-, *p*-XSC	DMBA	Female, SD rat, mammae	I	[47]
DASe	DMBA	Female, SD rat, mammae	NE	[48]
RSeCN	DMBA	Female, SD rat, mammae	I	[66]
BSC	AOM	Male, F344 rat, colon	I	[44]
p-XSC	NNK	Female, A/J mouse	I	[51]
		Male, F344 rat, lung and liver		
Selenite, selenate	DMAB	Male, F344 rat, colon	I	[52]
		Male, F344 rat, liver	NE	
Selenite	2-AAF	Male, CD rat, liver	I	[55]
Selenite	AFB_1	Male, F344 rat, liver	I	[49,50]
		Male/female, chicken, liver	E	

[a] DMBA: 7,12-dimethylbenz(*a*)anthracene; AOM: azoxymethane; NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; DMAB: 3,2′-dimethyl-4-aminobiphenyl; 2-AAF: 2-acetylaminofluorene; BOP: bis(2-oxopropyl)-nitrosamine; AFB_1: aflatoxin B_1.

[b] I: inhibition; NE: no effect; E: enhancement. Covalent DNA adducts derived from DMBA analyzed were *anti*-dG, *syn*-dA and *anti*-dA; O^6-mGu and 7-mGu derived from AOM or NNK were measured; two adducts derived from DMAB (C_8-dG and N_2-dG) were analyzed; in the case of 2-AAF, the total amount of [^{14}C]2-AAF covalently bound to liver DNA was quantified.

adduct formation and, thus, initiate carcinogenesis [59–61]. Covalent DNA adducts derived from DMBA that were analyzed in these studies were *anti*-diol-epoxide-deoxyguanosine (*anti*-dG), *syn*-diol-epoxide-deoxyadenosine (*syn*-dA), and *anti*-diol-epoxide-deoxyadenosine (*anti*-dA) [62]. They occurred in both the target (mammary epithelial tissue) and the non-target organ (liver). Daniel and Joyce [63] and Singletary [64] have shown that, following DMBA administration, *anti*-dG is the major adduct formed in rat mammary tissue in vivo. Maximum DNA binding in the mammary tissue of Sprague–Dawley rats was detected 24–48 h after DMBA administration.

Various forms of selenium have been shown to inhibit the initiation phase of carcinogenesis. Therefore, several investigators have examined the effect of selenite on carcinogen metabolism and carcinogen–DNA binding (Table 2). Dietary 1,4-phenylenebis-(methylene)selenocyanate (*p*-XSC) clearly inhibits total DMBA–DNA binding as quantified by assessing total tritium bound to the DNA of the mammary tissue [45]. The inhibition was confirmed by our finding of reduced levels of each of the three major adducts derived from DMBA [45]. Our results with *p*-XSC appear to be consistent with those reported by Milner and co-workers who employed selenite in their investigations [40–42,54]; the inhibitory effect by *p*-XSC is pronounced at early time points (6–48 h); it is not clear why it does not persist beyond 48 h. The fact that *p*-XSC inhibits mammary tumors induced by DMBA emphasizes that early time points (6–48 h) are critical for chemoprevention. A depression in the formation of the two major *anti*-diol-epoxide–deoxyribonucleoside adducts was reported by Ejadi et al. [41] after incubation of DMBA with mammary epithelial cells obtained from rats pretreated with selenite in vivo. Liu et al. [40,42] observed that selenite supplementation inhibited both *anti*- and *syn*-DMBA–DNA adducts in the mammary tissue in vivo. However, Ip and Daniel [43] reported that supplementation with selenite had no effect on levels of DMBA–DNA binding in the mammary tissue nor in the liver of rats in vivo; due to the limited amount of DNA obtained from the mammary tissue, the levels of individual adducts were not measured. Comparison of diallyl selenide (DASe) with its sulfur analog, diallyl sulfide (DAS), clearly demonstrates that the former is a superior inhibitor of DMBA-induced mammary tumors in the rat [48];

however, DASe had no effect on DMBA–DNA adduct formation so that the mechanism of mammary cancer prevention by DASe needs to be explored.

The form of selenium is important and determines its efficacy and toxicity in preclinical investigations. Thus, in an investigation of structure: activity relationship, we found that *o*-XSC was a more effective inhibitor of DMBA–DNA adduct formation in the rat mammary gland than *m*- and *p*-XSC; however, *p*-XSC was the least toxic [47]. To examine the biochemical basis for the inhibition of DMBA–DNA adduct formation, Sohn et al. determined the effect of *o*-, *m*- and *p*-XSC on both phase I and II enzymes [65]; they found that the effects on these enzymes vary depending on the isomer being examined. These findings may, in part, explain the protective effects of these isomers on adduct formation. On the other hand, inhibition of DMBA–DNA adduct formation in the rat mammary gland is not only caused by aromatic selenocyanate but also be aliphatic selenocyanates [66].

1.2.2. Lung tissue

Complete cessation of cigarette smoking would be the most effective way to prevent the lung cancer epidemic. However, it is recognized that a substantial number of smokers are unable to quit and thus, chemoprevention might be a possible way to mitigate the impact of smoking [67]. Our laboratory has been actively involved in the development of organoselenium chemopreventive compounds that can inhibit lung cancer in model studies; we used 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) as a representative tobacco-specific nitrosamine to induce lung tumors in rodents (Table 2). The inhibitory effect of *p*-XSC and selenite on NNK-induced DNA modification was measured by determining O^6-methylguanine (O^6-mGu) and 7-methylguanine (7-mGu) in lungs and livers of female A/J mice and male F344 rats [51]. The results clearly indicate that *p*-XSC is superior to selenite as an inhibitor of O^6-mGu and 7-mGu formation. This is consistent with the compounds' relative efficacy toward lung tumor inhibition in mice and suggests that *p*-XSC may inhibit NNK-induced lung tumors in the rat. Whether reduction of lung cancer in the study conducted by Clark et al. [38] that employed selenium-enriched yeast is due to reduction in levels of genetic damage needs to be determined. Currently, only selenium-enriched yeast is approved by the US Food and Drug Administration as a supplement for human usage. Approval for other forms of selenium awaits more detailed preclinical investigations.

1.2.3. Colon and liver tissues

In laboratory animals, both azoxymethane (AOM) and 3,2′-dimethyl-4-aminobiphenyl (DMAB) are generally used to induce colon cancer. Neither of the carcinogens is known to be present as environmental pollutants. Thus, etiological agents that are responsible for the development of colon cancer remain unidentified. Davis et al. examined the effect of selenite, selenate, and selenomethionine on DNA adduct formation with DMAB in rat, colon, and liver of F344 rats [52]. While selenite and selenate inhibited adduct formation in the rat colon, both had no effect on adduct formation in the liver. It is intriguing that selenomethionine even enhanced the levels of DMAB–DNA adducts [52]. Wortzman et al. [55] showed that selenite inhibited DNA adduct formation with 2-acetylaminofluorene (a liver carcinogen) in rat liver. Similar findings were observed with aflatoxin, another liver carcinogen. In contrast, selenite enhanced aflatoxin-induced DNA adduct formation in chicken liver [49,50]. The results indicate that genetic damage or protection depends largely on the form, as well as the levels, of selenium and the type of species employed. These results underscore that caution should be exercised when extending our knowledge from rodents to humans.

Fiala et al. [44] have shown that diet supplementation with benzyl selenocyanate (BSC, an analogue of *p*-XSC) reduced the levels of O^6- and N-7 methylation of deoxyguanosine in the colon but not in the liver of rats treated with the colon carcinogen azoxymethane (AOM). This is in good agreement with the inhibitory action of BSC on AOM-induced colon carcinogenesis [68]. Further studies by Fiala et al. demonstrated that BSC increases oxidative metabolism of AOM in the liver; thus, reducing delivery of reactive methylazoxymethanol to the target organ (colon) via the bloodstream [44]; an effect of BSC on AOM metabolism can explain the inhibition of DNA methylation in the colon by BSC. At the present time, there is no information on whether the reduction in human colon and liver cancers [32,33,38,39] is due, in part, to the protective effect of selenium on genetic damage.

Collectively, in most cases described above, the observed inhibitory effects of selenium compounds on DNA adduct formation are consistent with their ability to inhibit the initiation phase of carcinogenesis in laboratory animals. The short-term assay for DNA adduct formation is valuable for evaluating and screening multiple novel selenium compounds for their ability to inhibit the initiation phase of carcinogenesis.

1.3. The protective role of selenium on oxidative damage

Free radicals are characterized by an unpaired electron that makes them highly reactive but short-lived. Oxidation during normal cellular metabolism leads to the formation of free radicals such as those derived from molecular oxygen. Copper and iron provide electrons to molecular oxygen, producing more than one species (superoxide, hydroxyl radical, and singlet oxygen) of radicals with varied reactivities; one of the most reactive species is hydroxyl radical. The formation of free radicals is not limited to normal cellular functions but can occur via many reactions, such as those happening upon exposure to certain chemicals, radiation (including ultraviolet light), cigarette smoke, air pollutants, inflammation, strenuous exercise, and high-fat diets. Exposure of a healthy cell to free radicals is known to damage structures and consequently to interfere with functions of enzymes and critical macromolecules. A free radical produced within a cell will seek to attract another electron from surrounding molecules within the cell to become paired and stable. However, the outcome of such interaction is the formation of other free radicals derived from components of nucleic acids, lipids, carbohydrates, and proteins. Over time, human and animal cells have developed certain defense mechanisms that provide protection against oxidative damage induced by radicals.

Certain agents (selenium, Vitamins E, C, and β-carotene among many others) can scavenge free radicals; certain enzymes (superoxide dismutase, catalase, and glutathione peroxidase) are also capable of inactivating free radicals. Representative examples are superoxide dismutase, an Mn- and Cu/Zn-containing enzyme present in mitochondria, and in the cytoplasm, respectively. Catalase is an iron-containing enzyme detected in peroxisomes. Glutathione peroxidase is a selenium-containing enzyme that is also effective in catalyzing the decomposition of hydrogen peroxides and lipid peroxides. A balance between the formation of free radicals and protection against cellular damages induced by these species is essential for normal cellular function. When such a balance is disrupted as a result of excessive generation of damaging species or low levels of antioxidants, a cell will enter a state of oxidative stress. Following exposure to oxidative stress, the cell either dies, or repairs the damage. However, if the damage persists, the cell will enter a state of genetic instability that can lead to chronic diseases, including cancer [69]. Free radicals react with polyunsaturated fatty acids to form several products, including aldehydes that can engage the functionality of amine groups in proteins to form Schiff's base. Aldehydes can also react with amino lipids. Free radical damage to proteins and sulfur-containing enzymes leads to inactivation of enzymes, including those functioning as antioxidants. Free radical damage to carbohydrates can alter certain receptor functions. Free radicals can also modulate certain transcriptional factors and gene expression. However, when free radicals are not excessive, they are known to contribute to healthy functions in human health and development.

Supplementation of in vitro and in vivo reactions in rodents with inorganic selenium or various forms of organoselenium compounds inhibited both chemically- and physically-induced oxidative damage. Results from such experiments are summarized in Table 3 [70–78]. Protection against oxidative damage by selenium has been proposed because it is a component of glutathione peroxidase and other selenium-containing enzymes [24,79]. However, evidence for the role of selenium-containing enzymes in cancer prevention is not, as yet, clearly defined; an excellent review by Ganther is available on this subject [79]. On the other hand, excessive intake of selenium may result in oxidative damage leading to genomic instability [18]. Studies reported in Table 3 also demonstrate that protection against such damage was improved when selenium was used in combination with other vitamins and minerals. Lipid peroxidation products, commonly measured as thiobarbituric acid-reactive species (TBARS), have also been implicated as mediators of oxidative damage of DNA that leads to the formation of oxidized DNA bases such as 8-hydroxydeoxyguanosine (8-OHdG) [80]. Oxidative damage of DNA has frequently

Table 3
The effect of selenium, individually and in combination with vitamins and minerals, on oxidative damage to DNA, lipids and proteins in rodents

Study no.	Form of selenium[a]	Carcinogen/damaging agent	Sex, species, and organ	Parameter measured (outcome[b])	Reference
1	Selenite	*t*-Butylhydroperoxide	Male, SD rat, multiple organs (in vitro)	TBARS (I)	[109]
2	Selenite	$CBrCl_3$, *t*-butylhydroperoxide	Male, SD rat, liver slices and homogenates (in vitro)	Oxidized heme protein (I), TBARS (I)	[70]
3	Selenite	$CBrCl_3$	Male, SD rat, multiple organs (in vivo)	Oxidized heme protein (I)	[77]
4	Selenite	Spontaneous oxidative reaction	Male, SD rat, liver and heart (in vivo)	Oxidized heme protein (I)	[78]
5	Ebselen	Fe^{2+}/ADP/ascorbate	Male, Wister rat, microsomes (in vitro)	TBARS (I)	[71]
6	Selenite	DMBA	Female, SD rat, mammary, liver (in vivo)	TBARS (I)	[72]
7	Selenite	AFB_1	Male, F344 rat, liver (in vivo)	8-OHdG (I)	[73]
8	BSC	2-NP	Male, F344 rat, liver DNA (in vivo)	8-OHdG (I)	[108]
9	*p*-XSC	NNK	Female, A/J mouse, lung; male, F344 rat, lung (in vivo)	8-OHdG (I)	[75]
10	*p*-XSC	DMBA	Female, CD rat, mammae (in vivo)	8-OHdG (I)	[110]
11	Selenite	BOP	Male, Syrian hamster, pancreas (in vivo)	SSB (I)	[74]
12	Selenite	Exhaustive physical exercise	Female, albino rat, lung	Free radical generation detected by ESR (I)	[76]
13	Ebselen, selenomethionine and selenocysteine	Peroxynitrite	In vitro (plasmid supercoiled DNA)	SSB (I)	[111]

[a] In study no. 1, selenite was used alone or in combination with Vitamin E, β-carotene, and coenzyme Q_{10}. In study no. 2, selenite was used alone or in combination with Vitamin E and β-carotene. In study no. 3, selenite was used alone or in combination with numerous antioxidants. In study no. 4, it was used in combination with Vitamin E and other antioxidants. In study nos. 5 and 6, ebselen was used with or without Vitamin E. In study no. 12, selenite was used in combination with Vitamin E. Better effect was achieved with the combination regimen in study nos. 3, 5, and 6.

[b] I: inhibition; 8-OHdG: 8-hydroxydeoxyguanosine; ESR: electron spin resonance; TBARS: thiobarbituric acid reactive species derived from lipid peroxidation; SSB: single strand breaks.

been implicated in the carcinogenesis process and 8-OHdG has emerged as a biologically relevant marker for cellular oxidative stress [81–84]. Representative chemical carcinogens such as the lung carcinogens found in tobacco, tobacco smoke, and other sources, namely NNK, benzo(*a*)pyrene (B*a*P), 2-nitropropane, the carcinogens like the food-derived, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline, and the food contaminant, aflatoxin, as well as the synthetic carcinogen DMBA have been shown to increase the levels of 8-OHdG in rodents [85–91].

Independent of its form, selenium inhibited formation of such a lesion (cf. Table 3). 8-OHdG is a mutagenic lesion that produces G–T transversion. The urinary excretion rate of 8-OHdG has been utilized as a biomarker of the rate of oxidative DNA damage [84,92]. Several methods have been developed for the quantitation of femtomol levels of 8-OHdG in cellular DNA. They include HPLC/EC, GC/MS, ^{32}P-postlabeling, and immunoassays [84,92–99]. The advantages and disadvantages of each method are described but the HPLC/ED method is used most frequently [98]. Additional reports have demonstrated that smokers excrete ~50% more 8-OHdG in urine than do non-smokers [100,101]; these observations appear consistent with the hypothesis that there is an increased level of oxidative stress in smokers. In line with these observations, smokers have lower concentrations of plasma antioxidants, suggesting a lower intake and/or a higher rate of turnover of antioxidants in smokers [102–105]. Levels of 8-OHdG were higher in human tumor tissue than in non-tumor tissue in the lung, colon, breast, ovary, stomach, and brain [106,107]. It is of significance that selenium has been shown to inhibit oxidative damage to lipids, proteins, and DNA in rodents, as described above [70–78,108–111]; similar studies in humans are scarce in the literature [36]. Whether reduction of lung, colon, and prostate cancers in the clinical trial that employed selenium-enriched yeast [38,39] is due, in part, to a reduction in levels of various kinds of oxidative damage, including 8-OHdG, remains to be determined.

1.4. *The effects of selenium on cell growth and molecular targets of carcinogenesis*

The mechanism by which selenium compounds inhibit tumor formation during the initiation phase has been explored in vitro and in well-defined animal models [19–22,112]. Oxidative damage has been implicated in the development of cancer during the initiation phase but more so during the promotion phase of carcinogenesis. Yet, the mechanisms that can actually account for chemoprevention by selenium during the promotion/progression phase of carcinogenesis need, as yet, to be fully explored.

The effects of selenium on cellular and molecular targets that are critical in cell growth inhibition and in carcinogenesis are summarized in Table 4 [113–151]. Initial studies by Medina, and later on by Thompson and co-workers, as well as studies conducted in our laboratory, clearly demonstrated that inorganic selenium compounds appear to cause distinctly different cellular effects from those elicited by organic forms of selenium (reviewed in [22]). Inorganic selenium compounds in cell culture systems at levels of 5–10 μM can induce single strand breaks in DNA, and cell death by necrosis. However, certain organoselenium compounds, even at higher levels of selenium (10–50 μM), can cause cell death by apoptosis without evidence of DNA single strand breaks. It has been shown that the chemopreventive effect of selenium is due in part to its inhibitory effect on cell growth, DNA, RNA, and protein synthesis in transformed cells [113–127]. Changes in stress-related cellular proteins have been implicated in explaining the protective effects of selenium [147]. Because protein kinases (PKC) play a central role in the regulation of cell growth, tumor promotion, and differentiation, several reports described the inhibitory effect of selenium on kinase activities [142,143,146,148]. Cell cycle cdk2 or cell signaling protein kinases and/or a number of redox-regulated proteins — including the critical transcriptional factors (AP-1 and NF-κB) — have been proposed as targets against which selenium exerts its chemopreventive effect [117,122,128–133]. Limited studies on the inhibition of cyclooxygenases by selenium are reported [141].

Studies with cell cultures suggest that selenium may exert its chemopreventive effect via induction of apoptosis and inhibition of cell growth in transformed cells [134–140]. Moreover, induction of the *p53* gene by selenium compounds was demonstrated but the induction of apoptosis may not be entirely due to the response of *p53*'s to selenium [117]. Fiala et al. [149], following the development of an improved assay to

Table 4
The effect of selenium on cell growth and molecular targets of carcinogenesis

Form of selenium	Parameter[a]	Outcome[b]	References
Selenite	Growth of Ehrlich ascites tumor cells in mice	I	[113]
Selenite	Growth of L1210 leukemic cells	I	[114]
Selenodiglutathione (SDG)	Growth of L1210 leukemic cells	I	[115,116]
Selenite, SDG	Cell growth (in vitro)	I	[118–120]
p-XSC, BSC, Selenite	DNA, RNA, and protein synthesis	I	[120,121]
	Apoptosis	E	
Selenite	DNA synthesis (in vitro)	I	[122,123]
Selenite	RNA and protein synthesis	I	[124]
	Cell death (necrosis SSB)	E	
Selenite	Cell growth	I	[125–127]
Selenite	Cell cycle	Block (S/G_2-M)	[122,128,129]
Selenite	*p53*	E	[117,130–133]
	AP-1	I	
	NF-κB	I	
CH_3SeCN, Se-methylselenocysteine, *p*-XSC	In vitro DNA synthesis	I	[134–140]
	Cell cycle	Block (G_1)	
	Apoptosis	E	
BSC and its glutathione conjugates	ACF	I	[141]
	COX-2	I	
p-XSC, BSC	PKC, PKA	I	[142]
Selenite	PKC	I	[143]
p-XSC	TK	I	[144]
Selenite, selenium dioxide, selenic acid	Phospholipid/Ca^{2+}-dependent PKC	I	[145]
Ebselen	PKC	I	[146]
p-XSC, BSC	JNK	Dose-dependent E/I	[147]
p-XSC, BSC, selenite	DNA cytosine methyl-transferase	I	[149]
Se-methylselenocysteine	PKC (in vitro)	I	[148]
Se-methylselenocysteine, triphenylselenium chloride	Cell proliferation and cell cycle biomarkers (in vivo)	I/E/NE	[150]
p-XSC	PKC and 8-isoprostane (in vivo)	I	[151]

[a] AP-1: activator protein 1; NF-κB: nuclear factor κB; ACF: aberrant crypt foci; COX-2: cyclooxygenase-2; PKC and PKA: protein kinase C and A; TK: thymidine kinase; JNK: Jun-*N*-kinase.
[b] I: inhibition; E: enhancement; W: weak; NE: no effect.

assess methyltransferase (Mtase) activity, suggested that inhibition of Mtase may be a major mechanism of chemoprevention by selenium compounds at the postinitiation phase of carcinogenesis. These investigators [149] showed that selenite, *p*-XSC and BSC inhibited Mtase extracted from a human colon carcinoma with IC_{50}'s of 3.8, 5.2 and 8.1 μM, respectively. *p*-XSC also inhibited the Mtase activity and growth of human colon carcinoma HTC 116 cells with an IC_{50} of ∼20 μM. Although various selenium compounds with diverse chemical structures are known to inhibit cell proliferation in vitro, little is known regarding selenium intake and its effect on cell proliferation in vivo in normal growing cells or in neoplastic cells of the same organ following carcinogen treatment. Toward this end, an excellent report by Ip et al., described that the effect of selenium on cell proliferation and cell cycle biomarkers varies depending on the form, and whether cells are normal or transformed [150]. Findings of this study suggest that early transformed cells are sensitive to selenium intervention, whereas normal cells are not [150]. In addition to noting an inhibitory effect of *p*-XSC in vitro, Rao et al. recently demonstrated in vivo that *p*-XSC is capable of inhibiting PKC activities in the colonic mucosa and in tumors of rats treated with AOM [151]. As discussed above, there are several plausible biomarkers that can be selected as targets in the design of future clinical chemoprevention intervention strategies. However, initial pilot studies are required to test and validate the

most appropriate biomarker that will be highly useful in the area of cancer prevention trials in the clinic.

1.5. Summary, suggested recommendation of selenium intake and future directions

Food is the major source of selenium intake but limited efforts at elucidating structures of organoselenium compounds in common foods have been made [152–156]. There is a need for further studies in this area. Supplementing dietary selenium intake has been the aim of few clinical trials in cancer prevention. Compounds that have, thus far, been identified in selenium-enriched yeast, that had been utilized in a successful human clinical intervention trial [38,39] are selenomethionine, Se-methylselenocysteine, selenocysteine, and selenoethionine; however, the form of selenium that is responsible for cancer prevention remains undefined. Thus, it is essential to determine which types of selenium compounds provide optimal protection against genetic damage with the least toxicity. The average Se intake of a US resident is about 70–100 μg per day. At this level the normal criteria for nutritional requirement are satisfied but they are not sufficient for cancer prevention [17]. The mean plasma selenium level in US residents is 100 ± 30 S.D. μg/g. Selenium intake in most parts of Europe is considered lower than in the US [17]. Intake of 200 μg Se per day has been proven safe in the clinic [38,39]. On the basis of extensive studies, it has been proposed that about 400 μg per day is considered an upper limit [17,112,157–159]. Despite concerns about the toxicity of higher dietary levels of selenium, humans consuming up to about 700 μg Se per day appear to have no adverse clinical symptoms [159,160].

The data in this report clearly show that the dose and formulation (structure) of selenium compounds are critical determinants with regard to cellular responses. Inorganic selenium compounds appear to cause distinctly different cellular effects from those elicited by organic forms of selenium in vitro and in vivo in preclinical and clinical investigations [22,161–166]. Clearly, selenium compounds are capable of inhibiting, carcinogen-induced covalent DNA adduct formation and DNA oxidative damage, DNA methylation, micronuclei induction, chromosomal aberrations, and cancer [20–22,148,161–166]. The bulk of our knowledge on the role of selenium on genetic stability is based primarily on animal data and from studies conducted in in vitro systems. Laboratory animals and in vitro assays have greatly aided our understanding of the mechanisms responsible for the protective role of selenium against genetic damage and cancer. How far such knowledge is applicable to humans remains unclear because there is convincing evidence that some features of selenium metabolism are unique to humans [167]. In addition, in vitro assays and preclinical studies have generally employed higher levels of selenium per kg body weight than those measured in human applications. Clearly, there is a need for pilot studies aimed at determining the role of various forms of selenium; especially those approved for human use on cellular and molecular targets that are critical in the multi-step carcinogenic process. These types of studies are necessary to learn whether data obtained in rodents and in vitro systems are applicable to humans.

It appears that the use of selenium in combination with other minerals and vitamins, especially Vitamin E, is a promising approach toward inhibiting genetic damage and cancer development. In contrast to selenite, the synthetic organoselenium compounds can be tailored to achieve greater chemopreventive efficacy with minimal toxic side effects by structural modifications [20]. Novel chemopreventive agents need to be developed and tested under defined protocols of carcinogenesis and anti-carcinogenesis. Evaluation of the efficacy of these compounds in inhibition of cancer in laboratory animals is needed before a realistic assessment of the potential of these compounds for human application is at hand. The structural and functional identification of selenium-containing proteins may also contribute to our understanding of the role of selenium compounds in cancer prevention. A comprehensive database of the toxicologic and pharmacologic properties of selenium compounds and their mechanisms of action will help to translate laboratory findings into human application.

Acknowledgements

We thank Mrs. Patricia Sellazzo for preparing this manuscript and Mrs. Ilse Hoffmann for editing it. The work performed in the author's laboratory and described in this manuscript was supported by the National Cancer Institute (USA), Grant nos. CA

46589, CA 70972 and DE 13222 from the National Institutes of Dental and Craniofacial Research (USA). This is manuscript no. 27 in the series: "Selenium in Chemoprevention of Carcinogenesis".

References

[1] E.L. Wynder, G.B. Gori, Contribution of the environment to cancer incidence: an epidemiologic exercise, J. Natl. Cancer Instit. 58 (1977) 825–832.

[2] R. Doll, Nature and nurture: possibilities for cancer control, Carcinogenesis 17 (1996) 177–184.

[3] R. Doll, The lessons of life: keynote address to the nutrition and cancer conference, Cancer Res. 52 (1992) 2024s–2029s.

[4] R. Doll, R. Peto, The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today, J. Natl. Cancer Instit. 66 (1981) 1191–1308.

[5] K. El-Bayoumy, F.-L. Chung, J. Richie Jr., B.S. Reddy, L. Cohen, J. Weisburger, E.L. Wynder, Dietary control of cancer, Proc. Soc. Exp. Biol. Med. 216 (1997) 211–223.

[6] W.C. Willett, Diet, nutrition and avoidable cancer, Environ. Health Perspect. 103 (Suppl. 8) (1995) 165–170.

[7] P. Saltman, J. Gurin, I. Mothner, The University of California San Diego Nutrition Book, Little, Brown & Company, Boston, 1993.

[8] G.F. Combs Jr., S.B. Combs, The nutritional biochemistry of selenium, Ann. Rev. Nutr. 4 (1984) 257–280.

[9] W.R. Wolf, A. Schubert, Foods, in: M. Inhant (Ed.), Occurrence and Distribution of Selenium, CRC Press, Boca Raton, FL, 1989, pp. 107–129.

[10] V.R. Young, A. Nahapetian, M. Janghorbani, Selenium bioavailability with reference to human nutrition, Am. J. Clin. Nutr. 35 (1982) 1076–1088.

[11] O.A. Levander, R.F. Burk, Selenium, in: E.E. Ziegler, L.J. Filer (Eds.), Present Knowledge in Nutrition, ILSI Press, Washington, DC, 1996.

[12] H.E. Ganther, Pathways of selenium metabolism including respiratory excretory products, J. Am. Coll. Toxicol. 5 (1986) 1–15.

[13] Institute of Medicine, Dietary reference intakes: calcium, phosphorus, magnesium, vitamin D and fluoride, Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, National Academy of Sciences, National Academy Press, Washington, DC, 1997.

[14] Institute of Medicine, Dietary reference intakes for thiamin: riboflavin, niacin, vitamin B_6, folate vitamin B_{12}, pantothenic acid, biotin, and choline, Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, National Academy of Sciences, National Academy Press, Washington, DC, 1998.

[15] National Research Council, Recommended dietary allowances, Subcommittee on the 10th Edition of the RDAs, Food and Nutrition Board, Commission on Life Sciences, National Academy of Sciences, National Academy Press, Washington, DC, 1989.

[16] J.W. Wilson, C.W. Enns, J.D. Goldman, K.S. Tippett, S.J. Mickle, L.E. Cleveland, P.S. Chahil, Data tables: combined results from USDA's 1994 and 1995 continuing survey of food intakes by individuals and 1994 and 1995 diet and health knowledge survey, USDA/ARS Food Surveys Research Group, Beltsville Human Nutrition Research Center, Riverdale, MD, 1997.

[17] M.P. Rayman, The importance of selenium to human health (review), Lancet 356 (2000) 233–241.

[18] J.E. Spallholz, On the nature of selenium toxicity and carcinostatic activity, Free Radical Biol. Med. 17 (1994) 45–64.

[19] C. Ip, Selenium inhibition of chemical carcinogenesis, Fed. Proc. 44 (1984) 2573–2578.

[20] K. El-Bayoumy, The role of selenium in cancer prevention, in: V.T. DeVita Jr., S. Hellman, S.A. Rosenberg (Eds.), Cancer: Principles and Practice of Oncology, Vol. 2, 4th Edition, Lippincott, Philadelphia, PA, 1999, pp. 1–15.

[21] G.F. Combs Jr., W.P. Gray, Chemopreventive agents: selenium, Pharmacol. Ther. 79 (1998) 179–192.

[22] C. Ip, Lessons from basic research in selenium and cancer prevention, J. Nutr. 128 (1998) 1845–1854.

[23] K. Schwartz, C. Foltz, Selenium as an integral part of factor 3 against necrotic liver degeneration, J. Am. Chem. Soc. 79 (1997) 3292–3293.

[24] J.T. Rotruck, A.L. Pope, H.E. Ganther, A.B. Swanson, D.G. Hafeman, W.G. Hoekstra, Selenium: biochemical role as a component of glutathione peroxidase, Science 179 (1973) 588–590.

[25] R.J. Shamberger, D.V. Frost, Possible protective effect of selenium against human cancer, Can. Med. Assoc. J. 100 (1969) 682.

[26] K. Yoshizawa, W.C. Willett, S.J. Morris, M.J. Stampfer, D. Spiegelman, E.B. Rimm, E. Giovannucci, Study of prediagnostic selenium level in toenails and the risk of advanced prostate cancer, J. Natl. Cancer Instit. 90 (1998) 1219–1224.

[27] K.J. Helzlsouer, A.J. Alberg, E.P. Norkus, J.S. Morris, S.C. Hoffman, G.W. Comstock, Prospective study of serum micronutrients and ovarian cancer, J. Natl. Cancer Instit. 88 (1996) 32–37.

[28] J.J. Strain, E. Bokje, P. Van't Veer, J. Coulter, C. Stewart, H. Logan, W. Odling-Smee, R.A. Spence, K. Steele, Thyroid hormones and selenium status in breast cancer, Nutr. Cancer 27 (1997) 48–52.

[29] M.W. Russo, S.C. Murray, J.I. Wurzelmann, J.T. Woosley, R.S. Sandler, Plasma selenium levels and the risk of colorectal adenomas, Nutr. Cancer 28 (1997) 125–129.

[30] V. Bhuvarahamurthy, N. Balasubramanian, S. Govindasamy, Effect of radiotherapy and chemoradiotherapy on circulating antioxidant system of human uterine cervical carcinoma, Mol. Cell. Biochem. 158 (1996) 17–23.

[31] W.D. Guo, A.W. Hsing, J.Y. Li, J.S. Chen, W.H. Chow, W.J. Blot, Correlation of cervical cancer mortality with reproductive and dietary factors, and serum markers in China, Int. J. Epidemiol. 23 (1994) 1127–1132.

[32] S.Y. Yu, J.J. Zhu, W.G. Li, Q.-S. Huang, C. Zhi-Huang, Q. Nan-Zang, C. Hou, A preliminary report on the intervention

trials of primary liver cancer in high-risk populations with nutritional supplementation of selenium in China, Biol. Trace Elem. Res. 29 (1991) 289–294.

[33] S.Y. Yu, Y.J. Zhu, W.G. Li, Protective role of selenium against hepatitis B virus and primary liver cancer in Qidong, Biol. Trace Elem. Res. 56 (1997) 117–124.

[34] W.J. Blot, J.Y. Li, P.R. Taylor, W. Guo, S. Dawsey, G.Q. Wang, C.S. Yang, S.F. Zheng, M. Gail, G.Y. Li, B. Yu, V. Liu, J. Tangrea, Y. Sun, F. Liu Jr., J.F. Fraumeni, Y.-H. Zhang, B. Li, Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population, J. Natl. Cancer Instit. 85 (1993) 1483–1492.

[35] J.Y. Li, P.R. Taylor, B. Li, S. Dawsey, G.Q. Wang, A.G. Ersho, W. Guo, S.F. Liu, C.S. Yang, Q. Shen, et al., Nutrition intervention trials in Linxian, China: multiple vitamin/mineral supplementation, cancer incidence, and disease-specific mortality among adults with esophageal dysplasia, J. Natl. Cancer Instit. 85 (1993) 1492–1498.

[36] M.P.R. Prasad, M.A. Mukundan, K. Krishnaswamy, Micronuclei and carcinogen DNA adducts as intermediate end points in nutrient intervention trial of precancerous lesions in the oral cavity, Eur. J. Cancer, Part B: Oral Oncol. 31B (1995) 155–159.

[37] L. Bonelli, Chemoprevention of metachronous adenomas of the large bowel by means of antioxidants: a double-blind randomized trial, in: Proceedings of the Presentation at the International Selenium Tellurium Development Association Meeting, Scottsdale, AZ, 1998.

[38] L.C. Clark, G.F. Combs Jr., B.W. Turnbull, E.H. Slate, D.K. Chalker, J. Chow, L.S. Davis, R.A. Glover, G.F. Graham, E.G. Gross, Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin: a randomized controlled trial, J. Am. Med. Assoc. 276 (1996) 1957–1963.

[39] L.C. Clark, B. Dalkin, A. Krongrad, G.F. Combs Jr., B.W. Turnbull, E.H. Slate, R. Witherington, J.H. Herlong, E. Janosko, D. Carpenter, C. Borosso, S. Falk, J. Rounder, Decreased incidence of prostate cancer with selenium supplementation: results of a double-blind cancer prevention trial, Br. J. Urol. 81 (1998) 730–734.

[40] J.Z. Liu, J.A. Milner, Influence of selenium, age, and dosage of 7,12-dimethylbenz(*a*)anthracene (DMBA) on the in vivo formation of DNA adducts in mammary tissue, FASEB J. 5 (1991) 3239.

[41] S. Ejadi, I.D. Bhattacharya, K. Voss, K. Singletary, J.A. Milner, In vitro and in vivo effects of sodium selenite on 7,12-dimethylbenz(*a*)anthracene–DNA adduct formation in isolated rat mammary epithelial cells, Carcinogenesis (London) 10 (1989) 823–826.

[42] J. Liu, K. Gilbert, H.M. Parker, W.M. Haschek, J.A. Milner, Inhibition of 7,12-dimethylbenz(*a*)anthracenebenz(*a*)anthracene-induced mammary tumors and DNA adducts by dietary selenite, Cancer Res. 51 (1991) 4613–4617.

[43] C. Ip, F.B. Daniel, Effects of selenium on 7,12-dimethylbenz(*a*)anthracene-induced mammary carcinogenesis and DNA adduct formation, Cancer Res. 45 (1985) 61–65.

[44] E.S. Fiala, C. Joseph, O.-S. Sohn, K. El-Bayoumy, B.S. Reddy, Mechanism of benzylselenocyanate inhibition of azoxymethane-induced colon carcinogenesis in F344 rats, Cancer Res. 51 (1991) 2826–2830.

[45] K. El-Bayoumy, Y.-H. Chae, P. Upadhyaya, C. Meschter, L.A. Cohen, B.S. Reddy, Inhibition of 7,12-dimethylbenz(*a*)anthracene-induced tumors and DNA adduct formation in the mammary glands of female Sprague–Dawley rats by the synthetic organoselenium compound, 1,4-phenylenebis(methylene)selenocyanate, Cancer Res. 52 (1992) 2402–2407.

[46] P. Upadhyaya, K. El-Bayoumy, Effect of dietary soy protein isolate, genistein, and 1,4-phenylenebis(methylene)-selenocyanate on DNA binding of 7,12-dimethylbenz(*a*)-anthracene in mammary glands of CD rats, Oncol. Rep. 5 (1998) 1541–1545.

[47] Y.-H. Chae, P. Upadhyaya, K. El-Bayoumy, Structure–activity relationships among the *ortho*-, *meta*- and *para*-isomers of phenylenebis(methylene)selenocyanate (XSC) as inhibitors of 7,12-dimethylbenz(*a*)anthracene–DNA binding in mammary glands of female CD rats, Oncol. Rep. 4 (1997) 1067–1071.

[48] K. El-Bayoumy, Y.-H. Chae, P. Upadhyaya, C. Ip, Chemoprevention of mammary cancer by diallyl selenide, a novel organoselenium compound, Anticancer Res. 16 (1996) 2911–2916.

[49] J. Chen, M.P. Goetchius, T.C. Campbell, G.F. Combs Jr., Effects of dietary selenium and vitamin E on hepatic mixed-function oxidase activities and in vivo covalent binding of aflatoxin B_1 in rats, J. Nutr. 112 (1982) 324–331.

[50] J. Chen, M.P. Goetchius, G.F. Combs Jr., C. Campbell, Effects of dietary selenium and vitamin E on covalent binding of aflatoxin to chick liver cell macromolecules, J. Nutr. 112 (1982) 350–355.

[51] B. Prokopczyk, J.E. Cox, P. Upadhyaya, S. Amin, D. Desai, D. Hoffmann, K. El-Bayoumy, Effects of dietary 1,4-phenylenebis(methylene)selenocyanate on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced DNA adduct formation in lung and liver of A/J mice and F344 rats, Carcinogenesis 17 (1996) 749–753.

[52] C.D. Davis, Y. Feng, D.W. Hein, J.W. Finley, The chemical form of selenium influences 3,2′-dimethyl-4-aminobiphenyl–DNA adduct formation in rat colon, J. Nutr. 129 (1999) 63–69.

[53] T. Lawson, D.F. Birt, Enhancement of the repair of carcinogen-induced DNA damage in the hamster pancreas by dietary selenium, Chem. Biol. Interact. 45 (1983) 95–104.

[54] J.-Z. Liu, J.A. Milner, Age, dietary selenium and quantity of 7,12-dimethylbenz(*a*)anthracene influence the in vivo occurrence of rat mammary DNA adducts, J. Nutr. 122 (1992) 1361–1368.

[55] M.S. Wortzman, H.J. Besbris, A.M. Cohen, Effect of dietary selenium on the interaction between 2-acetylaminofluorene and rat liver DNA in vivo, Cancer Res. 40 (1980) 2670–2676.

[56] J.A. Milner, M.A. Pigott, A. Dipple, Selective effects of selenium on 7,12-dimethylbenz(*a*)anthracene–DNA binding in fetal mouse cell cultures, Cancer Res. 45 (1985) 6345–6347.
[57] C. Huggins, L.C. Grand, F.P. Brillantes, Mammary cancer induced by a single feeding of polynuclear hydrocarbons and its suppression, Nature (London) 189 (1961) 204–207.
[58] C.W. Welsch, Host factors affecting the growth of carcinogen-induced rat mammary carcinomas: a review and tribute to Charles Brenton Huggins, Cancer Res. 45 (1985) 3415–3443.
[59] A. Dipple, S.C. Cheng, C.A. Bigger, Polycyclic aromatic hydrocarbon carcinogens, Progr. Clin. Biol. Res. 347 (1990) 109–127.
[60] S.T. Vater, D.M. Baldwin, D. Warshawsky, Hepatic metabolism of 7,12-dimethylbenz(*a*)anthracene in male, female, and ovariectomized Sprague–Dawley rats, Cancer Res. 51 (1991) 492–498.
[61] C.J. Moore, W.A. Tricomi, M.N. Gould, Comparison of 7,12-dimethylbenz(*a*)anthracene metabolism and DNA binding in mammary epithelial cells from three rat strains with differing susceptibilities to mammary carcinogenesis, Carcinogenesis (London) 9 (1988) 2099–2102.
[62] K.W. Singletary, H.M. Parker, J.A. Milner, Identification and in vivo formation of ^{32}P-postlabeled rat mammary DMBA–DNA adducts, Carcinogenesis (London) 11 (1990) 1959–1963.
[63] F.B. Daniel, N.J. Joyce, DNA adduct formation by 7,12-dimethylbenz(*a*)anthracene and its noncarcinogenic 2-fluoro analogue in female Sprague–Dawley rats, J. Natl. Cancer Instit. 70 (1983) 111–118.
[64] K.W. Singletary, Effect of dietary butylated hydroxytoluene on the in vivo distribution, metabolism, and DNA-binding of 7,12-dimethylbenz(*a*)anthracene, Cancer Lett. 49 (1990) 187–193.
[65] O.-S. Sohn, E.S. Fiala, P. Upadhyaya, Y.-H. Chae, K. El-Bayoumy, Comparative effects of phenylebis(methylene)selenocyanate isomers on xenobiotic metabolizing enzymes in organs of female CD rats, Carcinogenesis 20 (1999) 615–621.
[66] C. Ip, S. Vadhanavikit, H. Ganther, Cancer chemoprevention by aliphatic selenocyanates: effect of chain length on inhibition of mammary tumors and DMBA adducts, Carcinogenesis 16 (1995) 35–38.
[67] K. El-Bayoumy, D. Hoffmann, Nutrition and tobacco-related cancer, in: D. Heber, G. Blackburn (Eds.), Nutritional Oncology, Academic Press, San Diego, CA, 1999, pp. 299–324.
[68] B.S. Reddy, S. Sugie, H. Maruyama, K. El-Bayoumy, P. Marra, Chemoprevention of colon carcinogenesis by dietary organoselenium, benzylselenocyanate, in F344 rats, Cancer Res. 47 (1987) 5901–5904.
[69] H.S. Garewal, Antioxidants and Disease Prevention, CRC Press, Boca Raton, FL, 1997.
[70] H. Chen, L.J. Pellett, H.J. Andersen, A.L. Tappel, Protection by vitamin E, selenium, and β-carotene against oxidative damage in rat liver slices and homogenate, Free Radical Biol. Med. 14 (1993) 473–482.
[71] V. Narayanaswami, H. Sies, Antioxidant activity of ebselen and related selenoorganic compounds in microsomal lipid peroxidation, Free Radical Res. Commun. 10 (1990) 237–244.
[72] H. Takada, T. Hirooka, T. Hatano, Y. Hamada, M. Yamamoto, Inhibition of 7,12-dimethylbenz(*a*)anthracene-induced lipid peroxidation and mammary tumor development in rats by vitamin E in conjunction with selenium, Nutr. Cancer 17 (1992) 115–122.
[73] H.-M. Shen, C.-N. Ong, B.-L. Lee, C.-Y. Shi, Aflatoxin B_1-induced 8-hydroxydeoxyguanosine formation in rat hepatic DNA, Carcinogenesis 16 (1995) 419–422.
[74] T. Lawson, D.F. Birt, Enhancement of the repair of carcinogen-induced DNA damage in the hamster pancreas by dietary selenium, Chem.-Biol. Interact. 45 (1983) 95–104.
[75] J.G.V. Rosa, B. Prokopczyk, D.H. Desai, S.G. Amin, K. El-Bayoumy, Elevated 8-hydroxy-2′-deoxyguanosine levels in lung DNA of A/J mice and F344 rats treated with 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and inhibition by dietary 1,4-phenylenebis(methylene)selenocyanate, Carcinogenesis 19 (1998) 1783–1788.
[76] K.V. Reddy, T.C. Kumar, M. Prasad, P. Reddanna, Pulmonary lipid peroxidation and antioxidant defenses during exhaustive physical exercise: the role of vitamin E and selenium, Nutrition 14 (1998) 448–451.
[77] H. Chen, A.L. Tappel, Protection of vitamin E, selenium, trolox C, ascorbic acid palmitate, acetylcysteine, coenzyme Q_0, coenzyme Q_{10}, β-carotene, canthaxanthin, and (+)-catechin against oxidative damage to rat blood and tissues in vivo, Free Radical Biol. Med. 18 (1995) 949–953.
[78] C.L. De Mulder, H.T. Madabushi, A.L. Tappel, Protection by vitamin E, selenium, trolox, ascorbic acid palmitate, acetylcysteine, coenzyme Q, β-carotene, and (+)-catechin against oxidative damage to rat liver and heart tissue slices measured by oxidized heme proteins, J. Nutr. Biochem. 6 (8) (1995) 452–458.
[79] H.E. Ganther, Selenium metabolism, selenoproteins and mechanisms of cancer prevention: complexities with thioredoxin reductase, Carcinogenesis 20 (1999) 1657–1666.
[80] J.-W. Park, R.A. Floyd, Lipid peroxidation products mediate the formation of 8-hydroxydeoxyguanosine in DNA, Free Radical Biol. Med. 12 (1992) 245–250.
[81] B.N. Ames, Endogenous oxidative DNA damage, aging, and cancer, Free Radical Res. Commun. 7 (1989) 121–128.
[82] R.A. Floyd, J.J. Watson, J. Harris, M. West, P.K. Wong, Formation of 8-hydroxydeoxyguanosine, hydroxyl free radical adduct of DNA in granulocytes exposed to the tumor promoter, tetradecanoylphorbol acetate, Biochem. Biophys. Res. Commun. 137 (1986) 841–846.
[83] M.K. Shigenaga, J.-W. Park, K.C. Cundy, C.J. Gimeno, B.N. Ames, In vivo oxidative DNA damage: measurement of 8-hydroxy-2′-deoxyguanosine in DNA and urine by high-performance liquid chromatography with electrochemical detection, Methods Enzymol. 186 (1990) 521–530.
[84] R.A. Floyd, J.J. Watson, P.K. Wong, D.H. Altmiller, R.C. Rikard, Hydroxyl free radical adduct of deoxyguanosine: sensitive detection and mechanism of formation, Free Radical Res. Commun. 1 (1986) 163–172.

[85] T. Kato, R. Hasegawa, D. Nakae, M. Hirose, M. Yaono, L. Cui, Y. Kobayashi, Y. Konishi, N. Ito, T. Shirai, Dose-dependent induction of 8-hydroxyguanine and preneoplastic foci in rat liver by a food-derived carcinogen, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline, at low dose levels, Jpn. J. Cancer Res. 87 (1996) 127–133.

[86] H.-M. Shen, C.-N. Ong, B.-L. Lee, C.-Y. Shi, Aflatoxin B_1-induced 8-hydroxydeoxyguanosine formation in rat hepatic DNA, Carcinogenesis 16 (1995) 419–422.

[87] F.-L. Chung, Y. Xu, Increased 8-oxodeoxyguanosine levels in lung DNA of A/J mice and F344 rats treated with the tobacco-specific nitrosamines 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, Carcinogenesis 13 (1992) 1269–1272.

[88] M.A. Sipowicz, S. Amin, D. Desai, K.S. Kasprzak, L.M. Anderson, Oxidative DNA damage in tissues of pregnant female mice and fetuses caused by the tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), Cancer Lett. 117 (1997) 87–91.

[89] R.J. Mauthe, V.M. Cook, S.L. Coffing, W.M. Baird, Exposure of mammalian cell cultures to benzo(*a*)pyrene and light results in oxidative DNA damage as measured by 8-hydroxydeoxyguanosine formation, Carcinogenesis 16 (1995) 133–137.

[90] K. Frenkel, L. Wei, H. Wei, 7,12-Dimethylbenz(*a*)anthracene induces oxidative DNA modification in vivo, Free Radical Biol. Med. 19 (1995) 373–380.

[91] E.S. Fiala, C.C. Conaway, J.E. Mathis, Oxidative DNA and RNA damage in the livers of Sprague–Dawley rats with the hepatocarcinogen 2-nitropropane, Cancer Res. 49 (1989) 5518–5522.

[92] M.K. Shigenaga, C.J. Gimeno, B.N. Ames, Urinary 8-hydroxy-2′-deoxyguanosine as a biological marker of in vivo oxidative DNA damage, Proc. Natl. Acad. Sci. U.S.A. 86 (1989) 9697–9701.

[93] S. Loft, A. Fischer-Nielsen, I.B. Jeding, K. Vistisen, H.E. Poulsen, 8-Hydroxydeoxyguanosine as a urinary biomarker of oxidative DNA damage, J. Toxicol. Environ. Health 40 (1993) 391–404.

[94] M. Dizdaroglu, Formation of an 8-hydroxyguanine moiety in deoxyribonucleic acid on γ-irradiation in aqueous solution, Biochemistry 24 (1985) 4476–4481.

[95] J.T. Lutgerink, E. de Graaf, J.B. Hoebee, H.F.C. Stavenuitez, J.G. Westra, E. Kriek, Detection of 8-hydroxyguanine in small amounts of DNA by ^{32}P-postlabeling, Anal. Biochem. 201 (1992) 127–133.

[96] P. Degan, M.K. Shigenaga, E.M. Park, P.E. Alperin, B.N. Ames, Immunoaffility isolation of urinary 8-hydroxyguanine-2′-deoxyguanosine and 8-hydroxyguanine and quantitation of 8-hydroxy-2′-deoxyguanosine in DNA by polyclonal antibodies, Carcinogenesis (London) 12 (1991) 865–871.

[97] J. Musarrat, A.A. Wani, Quantitative immunoanalysis of promutagenic 8-hydroxy-2′-deoxyguanosine in oxidized DNA, Carcinogenesis (London) 15 (1994) 2037–2043.

[98] B. Halliwell, M. Dizdaroglu, Commentary: the measurement of oxidative damage to DNA by HPLC and GC/MS techniques, Free Radical Res. Commun. 16 (1992) 75–87.

[99] A. Yarborough, Y.-J. Zhang, T.-M. Hsu, R.M. Santella, Immunoperoxidase detection of 8-hydroxyguanosine in aflatoxin B_1-treated rat liver and human oral mucosal cells, Cancer Res. 56 (1996) 683–688.

[100] H. Kiyosawa, M. Suko, H. Okudaira, K. Murata, T. Miyamoto, M.-H. Chung, H. Kasai, S. Nishimura, Cigarette smoking induces formation of 8-hydroxydeoxyguanosine, one of the oxidative DNA damages in human peripheral leukocytes, Free Radical Res. Commun. 11 (1990) 23–27.

[101] S. Loft, K. Vistisen, M. Ewertz, A. Tjonneland, K. Overvad, H.E. Poulsen, Oxidative DNA-damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index, Carcinogenesis (London) 13 (1992) 2241–2247.

[102] K. Murata, Smoking and vitamin C, World Rev. Nutr. Dietet. 64 (1991) 31–57.

[103] W.S. Stryker, L.A. Kaplan, E.A. Stein, M.J. Stampfer, A. Sober, W.C. Willett, The relation of diet, cigarette smoking, and alcohol consumption to plasma β-carotene and α-tocopherol levels, Am. J. Epidemiol. 127 (1988) 283–296.

[104] R.A. Riemersma, D.A. Wood, C.C. Macintyre, R.A. Elton, K.F. Gey, M.F. Oliver, Risk of angina pectoris and plasma concentrations of vitamins A, C, and E and carotene, Lancet 337 (1991) 1–5.

[105] J.T. Salonen, R. Salonen, K. Seppanen, et al., Effects of antioxidant supplementation on platelet function: a randomized pair-matched, placebo-controlled, double-blind trial in men with low antioxidant status, Am. J. Clin. Nutr. 53 (1991) 1222–1229.

[106] R. Olinski, T. Zastawny, J. Budzbon, J. Skokiwski, W. Zegarski, M. Dizdaroglu, DNA base modifications in chromatin of human cancerous tissues, Fed. Eur. Biochem. Soc. 309 (1992) 193–198.

[107] B. Yin, R.M. Whyatt, F.P. Perera, M.C. Randall, Y. Cooper, R.M. Santella, Determination of 8-hydroxyguanosine by immunoaffinity chromatography-monoclonal antibody-based ELISA, Free Radical Biol. Med. 18 (1995) 1023–1032.

[108] E.S. Fiala, O.-S. Sohn, H. Li, K. El-Bayoumy, R.S. Sodum, Inhibition of 2-nitropropane-induced rat liver DNA and RNA damage by benzyl selenocyanate, Carcinogenesis 18 (1997) 1809–1815.

[109] B. Leibowitz, M.-L. Hu, A.L. Tappel, Dietary supplements of vitamin E, β-carotene, coenzyme Q_{10} and selenium protect tissues against lipid peroxidation in rat tissue slices, Am. Instit. Nutr. May (1989) 97–104.

[110] K. El-Bayoumy, Y.-H. Chae, J.G. Rosa, L.K. Williams, D. Desai, S. Amin, E. Fiala, The effects of 1-nitropyrene, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine and 7,12-dimethylbenz(*a*)anthracene on 8-hydroxy-2′-deoxyguanosine levels in the rat mammary gland and modulation by dietary 1,4-phenylenebis(methylene)selenocyanate, Cancer Lett. 151 (2000) 7–13.

[111] I. Roussyn, K. Briviba, H. Masumoto, H. Sies, Selenium-containing compounds protect DNA from single-strand breaks caused by peroxynitrite, Arch. Biochem. Biophys. 330 (1996) 216–218.

[112] G.F. Combs Jr., L.C. Clark, in: D. Heber, G.L. Blackburn, V.L.W. Go (Eds.), Selenium and Cancer, Academic Press, 1999, Chapter 16, pp. 215–222.

[113] G.A. Greeder, J.A. Milner, Factors influencing the inhibitory effect of selenium on mice inoculated with Ehrlich ascites tumor cells, Science 209 (1980) 825–827.

[114] J.A. Milner, C.Y. Hsu, Inhibitory effects of selenium on the growth of L1210 leukemic cells, Cancer Res. 41 (1983) 1652–1656.

[115] K.A. Poirier, J.A. Milner, Factors influencing the antitumorigenic properties of selenium in mice, J. Nutr. 113 (1983) 2147–2154.

[116] A.M. Watrach, J.A. Milner, M.A. Watrach, Effect of selenium on growth rate of canine mammary carcinoma cells in athymic nude mice, Cancer Lett. 15 (1982) 137–143.

[117] J. Lanfear, J. Fleming, L. Wu, G. Webster, P.R. Harrison, The selenium metabolite selenodiglutathione induces *p53* and apoptosis: relevance to the chemopreventive effects of selenium? Carcinogenesis 15 (1994) 1387–1392.

[118] L. Wu, J. Lanfear, P.R. Harrison, The selenium metabolite selenodiglutathione induces cell death by a mechanism distinct from H_2O_2 toxicity, Carcinogenesis 16 (1995) 1579–1584.

[119] H.J. Thompson, A. Wilson, J. Lu, M. Singh, C. Jiang, P. Upadhyaya, K. El-Bayoumy, C. Ip, Comparison of the effects of an organic and inorganic form of selenium on a mammary carcinoma cell line, Carcinogenesis 15 (1994) 183–186.

[120] Z. Ronai, J.K. Tillotson, F. Traganos, Z. Darynkiewicz, C.C. Conaway, P. Upadhyaya, K. El-Bayoumy, Effects of organic and inorganic selenium compounds on rat mammary tumor cells, Int. J. Cancer 63 (1995) 428–434.

[121] J. Lu, C. Jiang, M. Kaeck, H. Ganther, C. Ip, H. Thompson, Cellular and metabolic effects of triphenylselenonium chloride in a mammary cell cultured model, Carcinogenesis 16 (1995) 513–517.

[122] D. Medina, C.J. Oborn, Selenium inhibition of DNA synthesis in mouse mammary epithelial cell line YN-4, Cancer Res. 44 (1984) 4361–4365.

[123] R. Cox, Selenite: a good inhibitor of rat liver DNA methylase, Biochem. Int. 10 (1985) 63–69.

[124] X.R. Jiang, M. Macey, H.X. Lin, A.C. Newland, The antileukaemic effects and the mechanism of sodium selenite, Leuk. Res. 16 (1992) 347–352.

[125] C. Ip, M. Ip, U. Kim, Dietary selenium intake and growth of the MT-W9B transplantable rat mammary tumor, Cancer Lett. 14 (1981) 101–107.

[126] M.E. Fico, K.A. Poirier, A.M. Watrach, J.A. Milner, Differential effects of selenium on normal and neoplastic canine mammary cells, Cancer Res. 46 (1986) 3384–3388.

[127] D. Medina, H. Lane, C.J. Oborn, Uptake and localization of selenium-75 in mammary epithelial cells in vitro, Cancer Lett. 15 (1982) 301–310.

[128] Z. Zhu, M. Kimura, Y. Itokawa, T. Aoki, J.A. Takahasi, S. Nakatsu, Y. Oda, H. Kikuchi, Apoptosis induced by selenium in human glioma cell lines, Biol. Trace Elem. Res. 54 (1996) 123–134.

[129] Z. Zhu, M. Kimura, Y. Itokawa, S. Nakatsu, Y. Oda, H. Kikuchi, Effect of selenium on malignant tumor cells in brain, Biol. Trace Elem. Res. 49 (1995) 1–7.

[130] B.C. Pence, M. Stewart, L. Walsh, G. Cameron, Modulation of oxidative damage to DNA be sodium selenite via the mechanism of apoptosis, in: Proceedings of the 6th International Symposium on Selenium in Biology and Medicine, Beijing, International Life Sciences Institute, Washington, DC, 1996, pp. 82–88.

[131] M. Meyer, R. Schreck, P.A. Baeuerle, H_2O_2 and antioxidants have opposite effects on activation of NF-κB and AP-1 in intact cells: AP-1 as secondary antioxidant responds faster, EMBO J. 12 (1993) 2005–2015.

[132] D. Galter, S. Mihm, W. Dröge, Distinct effects of glutathione disuphide on the nuclear transcription factor κB and the activator protein-1, Eur. J. Biochem. 221 (1994) 639–648.

[133] P.R. Harrison, J. Lanfear, L. Wu, J. Fleming, L. Blower, Mechanisms of chemoprevention and growth inhibition by selenium compounds, in: Proceedings of the 6th International Symposium on Selenium in Biology and Medicine, Beijing, International Life Sciences Institute, Washington, DC, 1996, pp. 74–82.

[134] R. Sinha, T. Said, D. Medina, Organic and inorganic selenium compounds inhibit mouse mammary cell growth in vitro by different cellular pathways, Cancer Lett. 107 (1996) 277–284.

[135] C. Jiang, J. Lu, G. Garcia, H.J. Thompson, Spontaneous nucleosomal DNA fragmentation in murine leukemic L1210 cells, Biochem. Biophys. Res. Commun. 194 (1993) 836–841.

[136] R. Sinha, D. Medina, Inhibition of cdk2 kinase activity by methylselenocysteine in synchronized mouse mammary epithelial tumor cells, Carcinogenesis 18 (1997) 1541–1547.

[137] J. Lu, M. Kaeck, C. Jiang, A.C. Wilson, H.J. Thompson, Selenite induction of DNA strand breaks and apoptosis in mouse leukemic L1210 cells, Biochem. Pharmacol. 49 (1994) 1531–1535.

[138] J. Lu, C. Jiang, M. Kaeck, H. Ganther, S. Vadhanavikit, C. Ip, H. Thompson, Dissociation of the genotoxic and growth inhibitory effects of selenium, Biochem. Pharmacol. 50 (1995) 213–219.

[139] J. Lu, H. Pei, C. Ip, D.J. Lisk, H. Ganther, H.J. Thompson, Effect of an aqueous extract of selenium-enriched garlic on in vitro markers and in vivo efficacy in cancer prevention, Carcinogenesis 17 (1996) 1903–1907.

[140] A.C. Wilson, H.J. Thompson, P.J. Schedin, N.W. Gibson, H.E. Ganther, Effect of methylated forms of selenium on cell viability and the induction of DNA strand breakage, Biochem. Pharmacol. 43 (1992) 1137–1141.

[141] Y. Kawamori, K. El-Bayoumy, B.-Y. Ji, J.G. Rosa Rodriguez, C.V. Rao, B.S. Reddy, Evaluation of benzyl selenocyanate glutathione conjugate for potential chemopreventive properties in colon carcinogenesis, Int. J. Oncol. 13 (1998) 29–34.

[142] P.G. Foiles, H. Fujiki, M. Suganuma, S. Okabe, J. Yatsunami, L.M. Miglietta, P. Upadhyaya, K. El-Bayoumy, Z. Ronai, Inhibition of PKC and PKA by chemopreventive organoselenium compounds, Int. J. Oncol. 7 (1995) 685–690.

[143] R. Gopalakrishna, Z.-H. Chen, U. Gundimeda, Selenocompounds induce a redox modulation of protein kinase C in the cell, compartmentally independent from cytosolic glutathione: its role in inhibition of tumor promotion, Arch. Biochem. Biophys. 348 (1997) 37–48.
[144] J.K. Tillotson, P. Upadhyaya, Z. Ronai, Inhibition of thymidine kinase in cultured mammary tumor cells by the chemopreventive organoselenium compound, 1,4-phenylenebis-(methylene)selenocyanate, Carcinogenesis 15 (1994) 607–610.
[145] H.D. Su, M. Shoji, G.J. Mazzei, W.R. Vogler, J.F. Kuo, Effects of selenium compounds on phospholipid/Ca^{2+}-dependent protein kinase (protein kinase C) system from human leukemic cells, Cancer Res. 46 (1986) 3684–3687.
[146] I.A. Cotgreave, S.K. Duddy, G.E. Kass, D. Thompson, P. Moldeus, Studies on the anti-inflammatory activity of ebselen. Ebselen interferes with granulocyte oxidative burst by dual inhibition of NADPH oxidase and protein kinase C? Biochem. Pharmacol. 38 (1989) 649–656.
[147] V. Adler, M.R. Pincus, S. Posner, P. Upadhyaya, K. El-Bayoumy, Z. Ronai, Effects of chemopreventive selenium compounds on Jun-*N*-kinase activities, Carcinogenesis 17 (1996) 1849–1854.
[148] R. Sinha, S.C. Kiley, J.X. Lu, H.J. Thompson, R. Moraes, S. Jaken, D. Medina, Effects of methylselenocysteine on PKC activity, cdk2 phosphorylation and *gadd* gene expression in synchronized mouse mammary epithelial tumor cells, Cancer Lett. 146 (1999) 135–145.
[149] E.S. Fiala, M.E. Staretz, G.A. Pandya, K. El-Bayoumy, S.R. Hamilton, Inhibition of DNA cytosine methyltransferase by chemopreventive selenium compounds determined by an improved assay for DNA cytosine methyltransferase and DNA cytosine methylation, Carcinogenesis 19 (1998) 597–604.
[150] C. Ip, H.J. Thompson, H.E. Ganther, Selenium modulation of cell proliferation and cell cycle biomarkers in normal and pre-malignant cells of the rat mammary gland, Cancer Epidemiol. Biomarkers Prev. 9 (2000) 49–54.
[151] C.V. Rao, B. Simi, Y. Hirose, P. Upadhyaya, K. El-Bayoumy, B.S. Reddy, Mechanisms in the chemoprevention of colon cancer: modulation of protein kinase C, tyrosine protein kinase and diacylglycerol kinase activities by 1,4-phenylenebis(methylene)selenocyanate and impact of low-fat diet, Int. J. Oncol. 16 (2000) 519–527.
[152] S.N. Nigan, W.B. McConnell, Seleno amino compounds from *Astragalus bisulcatus*: isolation and identification of γ-L-glutamyl-Se-methylseleno-L-cysteine and Se-methylseleno-L-cysteine, Biochem. Biophys. Acta 192 (1969) 185–190.
[153] X.J. Cat, E. Block, P.C. Uden, Z. Zhang, B.D. Quimby, J.J. Sullivan, *Allium* chemistry: identification of selenoamino acids in ordinary and selenium-enriched garlic, onion, and broccoli using gas chromatography with atomic emission detection, J. Agric. Food Chem. 43 (1995) 1754–1757.
[154] S.M. Bird, H. Ge, P.C. Uden, J.F. Tyson, E. Block, E. Denoyer, High-performance liquid chromatography of selenoamino acids and organoselenium compounds: speciation by inductively coupled plasma mass spectrometry, J. Chromatogr. A. 789 (1997) 349–359.
[155] P.C. Uden, S.M. Bird, M. Kotterbai, P. Nohbos, J.F. Tyson, E. Block, E. Denoyer, Analytical selenoamino acid studies by chromatography with interfaced atomic mass spectrometry and atomic emission spectral detection, Fresenius J. Anal. Chem. 362 (1998) 447–456.
[156] S.M. Bird, P.C. Uden, J.F. Tyson, E. Block, E. Denoyer, Speciation of selenoamino acids and organoselenium compounds in selenium-enriched yeast using high-performance liquid chromatography-inductively coupled plasma mass spectrometry, J. Anal. Atom Spectrom. 12 (1997) 785–788.
[157] National Research Council, Subcommittee on the 10th Edition of RDAs, National Academy Press, Washington, DC, 1989, p. 284.
[158] O.A. Levander, P.D. Whanger, Deliberations and evaluations of the approaches, endpoints and paradigms for selenium and iodine dietary recommendations, J. Nutr. 126 (Suppl. 9) (1996) 2427S–2434S.
[159] G. Yang, S. Yin, R. Zhou, L. Gu, B. Yan, Y. Liu, Y. Liu, Studies of safe maximal daily dietary intake in a seleniferous area in China. Part II. Relation between selenium intake and manifestations of clinical signs and certain biological altercations, J. Trace Elem. Electrolites Health Dis. 3 (1989) 123–130.
[160] M.P. Longnecker, P.R. Taylor, O.A. Levander, S.M. Howe, C. Veillon, P.A. McAdam, K.Y. Patterson, J.M. Holden, M.J. Stampfer, J.S. Morris, W.C. Willett, Selenium in diet, blood, and toenails in relation to human health in a seleniferous area, Am. J. Clin. Nutr. 53 (1991) 1288–1294.
[161] S. Biswas, G. Talukder, A. Sharma, Prevention of cytotoxic effects of arsenic by short-term dietary supplementation with selenium in mice in vivo, Mutat. Res. 441 (1999) 155–160.
[162] A.M. Khalil, A.O. Maslat, Chromosome aberrations, sister-chromatid exchanges and cell-cycle kinetics in human peripheral blood lymphocytes exposed to organoselenium in vitro, Mutat. Res. 232 (1990) 227–232.
[163] Y. Zhang, H. Xiao, Antagonistic effect of calcium, zinc and selenium against cadmium-induced chromosomal aberrations and micronuclei in root cells of *Hordeum vulgare*, Mutat. Res. 420 (1998) 1–6.
[164] W.Q. Chen, Effects of Se-enriched malt cakes on UV/benzo(*a*)pyrene-induced unscheduled DNA synthesis of lymphocytes from high risk population of lung cancer, Chin. J. Oncol. 14 (1992) 334–336.
[165] G.G. Hu, Investigation of protective effect of selenium on genetic materials among workers exposed to arsenic, Chin. J. Prev. Med. 23 (1989) 286–288.
[166] J. An, Q.G. Chen, F.Z. Gao, E. Zheng, Effect of Na_2SeO_3 on the damages of genetic materials induced by MNNG in children's foreskin fibroblasts in vitro, Chin. J. Oncol. 10 (1988) 180–183.
[167] P.D. Whanger, Metabolism of selenium in humans, J. Trace Elem. Exp. Med. 11 (1998) 227–240.