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
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 (T₃) [17]. A strong inverse relationship was observed between T₃ levels and cancer between the highest and lowest tertiles of intake in a study of postmenopausal breast cancer patients [28]; levels of selenium in toenails were positively associated with T₃ 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 lower 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

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

<sup>a</sup>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.

<sup>b</sup>In study nos. 1 and 2, populations were from Qideng, Shandong 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.

<sup>c</sup>I: inhibition; NE: no effect.
<table>
<thead>
<tr>
<th>Form of selenium</th>
<th>Carcinogen</th>
<th>Sex, species, organ</th>
<th>Outcome</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selenite</td>
<td>DMBA</td>
<td>Female, SD rat, mammae</td>
<td>I</td>
<td>[40–42]</td>
</tr>
<tr>
<td>Selenite</td>
<td>DMBA</td>
<td>Female, SD rat, mammae</td>
<td>NE</td>
<td>[43]</td>
</tr>
<tr>
<td>Selenite</td>
<td>DMBA</td>
<td>Female, SD rat, liver</td>
<td>NE</td>
<td></td>
</tr>
<tr>
<td>p-XSC</td>
<td>DMBA</td>
<td>Female, SD rat, mammae</td>
<td>I</td>
<td>[45,46]</td>
</tr>
<tr>
<td>p-XSC</td>
<td>DMBA</td>
<td>Female, SD rat, liver</td>
<td>NE</td>
<td></td>
</tr>
<tr>
<td>o-, m-, p-XSC</td>
<td>DMBA</td>
<td>Female, SD rat, mammae</td>
<td>I</td>
<td>[47]</td>
</tr>
<tr>
<td>DASe</td>
<td>DMBA</td>
<td>Female, SD rat, mammae</td>
<td>NE</td>
<td>[48]</td>
</tr>
<tr>
<td>RS4CN</td>
<td>DMBA</td>
<td>Female, SD rat, mammae</td>
<td>I</td>
<td>[66]</td>
</tr>
<tr>
<td>BSC</td>
<td>AOM</td>
<td>Male, F344 rat, colon</td>
<td>I</td>
<td>[44]</td>
</tr>
<tr>
<td>BSC</td>
<td>DMAB</td>
<td>Male, F344 rat, lung and liver</td>
<td>I</td>
<td>[51]</td>
</tr>
<tr>
<td>Selenite, selenate</td>
<td>DMAB</td>
<td>Male, F344 rat, colon</td>
<td>I</td>
<td>[52]</td>
</tr>
<tr>
<td>Selenite</td>
<td>2-AAF</td>
<td>Male, CD rat, liver</td>
<td>NE</td>
<td></td>
</tr>
<tr>
<td>Selenite</td>
<td>AFB1</td>
<td>Male, F344 rat, liver</td>
<td>I</td>
<td>[49,50]</td>
</tr>
</tbody>
</table>

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; AFB1: aflatoxin B1.
b I: inhibition; NE: no effect; E: enhancement. Covalent DNA adducts derived from DMBA analyzed were anti-dG, syn-dA and anti-dA; O6-mGu and 7-mGu derived from AOM or NNK were measured; two adducts derived from DMAB (C8-dG and N2-dG) were analyzed; in the case of 2-AAF, the total amount of [14C]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 \( \text{o}-\)XSC was a more effective inhibitor of DMBA–DNA adduct formation in the rat mammary gland than \( \text{m}-\) and \( \text{p}-\)XSC; however, \( \text{p}-\)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 \( \text{o}-\), \( \text{m}-\) and \( \text{p}-\)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 by 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 \( \text{p}-\)XSC and selenite on NNK-induced DNA modification was measured by determining \( \text{O}^6\)-methylguanine (\( \text{O}^6\)-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 \( \text{p}-\)XSC is superior to selenite as an inhibitor of \( \text{O}^6\)-mGu and 7-mGu formation. This is consistent with the compounds’ relative efficacy toward lung tumor inhibition in mice and suggests that \( \text{p}-\)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 \( \text{p}-\)XSC) reduced the levels of \( \text{O}^6\)- 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 transcription 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 thio-barbituric 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

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

<sup>a</sup> In study no. 1, selenite was used alone or in combination with Vitamin E, β-carotene, and coenzyme Q<sub>10</sub>. 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.

<sup>b</sup> 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 (BaP), 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

<table>
<thead>
<tr>
<th>Form of selenium</th>
<th>Parametera</th>
<th>Outcomeb</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selenite</td>
<td>Growth of Ehrlich ascites tumor cells in mice</td>
<td>I</td>
<td>[113]</td>
</tr>
<tr>
<td>Selenite</td>
<td>Growth of L1210 leukemic cells</td>
<td>I</td>
<td>[114]</td>
</tr>
<tr>
<td>Selenodiglutathione (SDG)</td>
<td>Growth of L1210 leukemic cells</td>
<td>I</td>
<td>[115,116]</td>
</tr>
<tr>
<td>Selenite, SDG</td>
<td>Cell growth (in vitro)</td>
<td>I</td>
<td>[118–120]</td>
</tr>
<tr>
<td>(p)-XSC, BSC, Selenite</td>
<td>DNA, RNA, and protein synthesis</td>
<td>I</td>
<td>[120,121]</td>
</tr>
<tr>
<td>Selenite</td>
<td>DNA synthesis (in vitro)</td>
<td>I</td>
<td>[122,123]</td>
</tr>
<tr>
<td>Selenite</td>
<td>RNA and protein synthesis</td>
<td>I</td>
<td>[124]</td>
</tr>
<tr>
<td>Selenite</td>
<td>Cell death (necrosis SSB)</td>
<td>E</td>
<td></td>
</tr>
<tr>
<td>Selenite</td>
<td>Cell growth</td>
<td>I</td>
<td>[125–127]</td>
</tr>
<tr>
<td>Selenite</td>
<td>Cell cycle</td>
<td>Block (S/G(_2)-M)</td>
<td>[122,128,129]</td>
</tr>
<tr>
<td>Selenite</td>
<td>(p)-XSC</td>
<td>E</td>
<td>[117,130–133]</td>
</tr>
<tr>
<td>CH(_3)SeCN, Se-methylselenocysteine, (p)-XSC</td>
<td>In vitro DNA synthesis</td>
<td>I</td>
<td>[134–140]</td>
</tr>
<tr>
<td>BSC and its glutathione conjugates</td>
<td>ACF</td>
<td>I</td>
<td>[141]</td>
</tr>
<tr>
<td>(p)-XSC, BSC</td>
<td>COX-2</td>
<td>I</td>
<td></td>
</tr>
<tr>
<td>Selenite</td>
<td>PKC, PKA</td>
<td>I</td>
<td>[142]</td>
</tr>
<tr>
<td>(p)-XSC</td>
<td>TK</td>
<td>I</td>
<td>[143]</td>
</tr>
<tr>
<td>Selenite, selenium dioxide, selenic acid</td>
<td>Phospholipid/Ca(^{2+})-dependent PKC</td>
<td>I</td>
<td>[145]</td>
</tr>
<tr>
<td>Ebselen</td>
<td>PKC</td>
<td>I</td>
<td>[146]</td>
</tr>
<tr>
<td>(p)-XSC, BSC</td>
<td>JNK</td>
<td>Dose-dependent E/I</td>
<td>[147]</td>
</tr>
<tr>
<td>(p)-XSC, BSC, selenite</td>
<td>DNA cytosine methyltransferase</td>
<td>I</td>
<td>[149]</td>
</tr>
<tr>
<td>Se-methylselenocysteine</td>
<td>PKC (in vitro)</td>
<td>I</td>
<td>[148]</td>
</tr>
<tr>
<td>Se-methylselenocysteine,</td>
<td>Cell proliferation and cell cycle biomarkers (in vivo)</td>
<td>I/E/NE</td>
<td>[150]</td>
</tr>
<tr>
<td>triphenylselenonium chloride</td>
<td>PKC and 8-isoprostane (in vivo)</td>
<td>I</td>
<td>[151]</td>
</tr>
</tbody>
</table>

\(\text{a}\) AP-1: activator protein 1; NF-\(\kappa\)B: nuclear factor \(\kappa\)B; ACF: aberrant crypt foci; COX-2: cyclooxygenase-2; PKC and PKA: protein kinase C and A; TK: thymidine kinase; JNK: Jun-\(\kappa\)-kinase.

\(\text{b}\) 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 \(\mu\)M, respectively. \(p\)-XSC also inhibited the Mtase activity and growth of human colon carcinoma HTCl 116 cells with an IC\(_{50}\) of ~20 \(\mu\)M. 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 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.

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