

Review

Selenium: From cancer prevention to DNA damage

Lucia Letavayová^a, Viera Vlčková^b, Jela Brozmanová^{a,*}

^a *Laboratory of Molecular Genetic, Cancer Research Institute, Slovak Academy of Sciences, 833 91 Bratislava, Slovak Republic*

^b *Department of Genetics, Faculty of Natural Sciences, Comenius University, 842 15 Bratislava, Slovak Republic*

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Abstract

Selenium (Se) is a dietary essential trace element with important biological roles. Accumulating evidence indicates that Se compounds possess anticancer properties. Se is specifically incorporated into proteins in the form of selenocysteine and non-specifically incorporated as selenomethionine in place of methionine. The effects of Se compounds on cells are strictly compositional and concentration-dependent. At supranutritional dietary levels, Se can prevent the development of many types of cancer. At higher concentrations, Se compounds can be either cytotoxic or possibly carcinogenic. The cytotoxicity of Se is suggested to be associated with oxidative stress. Accordingly, sodium selenite, an inorganic Se compound, was reported to induce DNA damage, particularly DNA strand breaks and base damage. In this review we summarize the various activities of Se compounds and focus on their relation to DNA damage and repair. We discuss the use of *Saccharomyces cerevisiae* for identification of the genes involved in Se toxicity and resistance.

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Keywords: Selenium; Free radicals; Toxicity; DNA damage; DNA repair

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* Corresponding author. Tel.: +421 2 59327 333; fax: +421 2 59327 350.
E-mail address: jela.brozmanova@savba.sk (J. Brozmanová).

1. Introduction

Selenium (Se) is a universal essential trace element for mammals which is important for many cellular processes. In the first half of the 20th century, Se, due to its toxicity was considered an undesirable element for higher organisms. Toxicity of Se was first confirmed in 1933 to occur in livestock that consumed plants of the genus *Astragalus*, *Xylorrhiza*, *Oonopsis* and *Stanleya* in the western regions of the United States. These plants have the ability to accumulate large quantities of Se from soil, and therefore are called Se accumulator plants, or Se indicator plants (Oldfield, 1987).

In the second half of the 20th century, a significant change in the importance of Se for human nutrition and biology took place. A new biological perspective of Se was shown by the pioneering work of Schwarz and Foltz (1957) who reported that Se at very low dietary concentrations is an essential nutrient. At low concentrations Se was able to prevent liver necrosis in rats consuming a Torula-yeast and Vitamin E deficient diet. Further support for the benefits of Se came after the discovery of the essential role of Se in the formation of glutathione peroxidase (Rotruck et al., 1973), thioredoxin reductase and other enzymes that provided protection against oxidative stress. After 1973 it was confirmed by numerous studies that selenoproteins and/or selenoenzymes were involved in the metabolism of all higher vertebrates. The accumulated evidence showing the role of Se in many areas important for human health has been reviewed by Rayman (2000).

Results obtained from epidemiological studies, laboratory bioassays and human clinical intervention supported a protective role(s) of Se against cancer development (Clark and Marshall, 2001; El-Bayoumy, 2001; Greenwald, 2004; Meuillet et al., 2004). However, the various organic and inorganic Se compounds used in such studies have produced mixed results when tested in animal models and human subjects. Studies performed *in vitro* have shown that both the dose and chemical form of Se compounds are critical factors in cellular responses (Ip, 1998). Se compounds at low concentration may have protective anticarcinogenic properties, whereas at higher concentration they can be genotoxic and possibly carcinogenic (Spallholz, 1994). The toxicity of Se compounds is now viewed as being caused by the generation of reactive oxygen species (ROS) (Kramer and Ames, 1988; Spallholz, 1997; Seko and Imura, 1997; Terada et al., 1999; Spallholz et al., 2004). In a manner similar to other ROS generating agents, some Se compounds may promote DNA oxidation *in vivo*. In accordance with the observations that Se generates ROS, sodium selenite

(SSe), an inorganic selenium containing compound, has been shown to induce DNA strand breaks in cell culture systems (Lu et al., 1994, 1995; Zhou et al., 2003).

It is evident that Se has multiple roles in biological systems. Many of them reside in its capability of acting as an antioxidant and disease preventing element. A number of excellent reviews have recently been written on the chemopreventive effects of Se (Rayman, 2000, 2005; El-Bayoumy, 2001; El-Bayoumy and Sinha, 2004, 2005; Whanger, 2004; Combs, 2005) and thus this topic will be addressed only very briefly. Se, depending upon chemical form, can be a prooxidant toxic agent that can induce DNA damage and cell death. The mechanisms that determine Se cytotoxicity and the induction of DNA damage are the main subject of present review.

2. Overview of Se in human health

As previously noted, Se is an essential dietary nutrient for all mammals. The initial US recommended daily allowance in 1989 was 50–70 $\mu\text{g}/\text{day}$ (this value has recently been lowered to 55 $\mu\text{g}/\text{day}$) for healthy human adults (El-Bayoumy, 2001; Whanger, 2004). This amount of Se may fulfill the dietary need for the 25 known (Stadtman, 2002) selenoproteins as well as for general human health (Rayman, 2000). Most of the human dietary Se requirement is met by dietary L-selenomethionine and, the lesser amounts of L-selenocysteine, both of which are components of animal proteins (Spallholz, 1994). Both organic selenomethionine (as a component of Se-enriched yeast) and inorganic SSe are commercial forms available as supplements (Shen et al., 2001).

Concerning the toxicity, Se has limited doses used in chemoprevention. Based on human studies, intakes of 400 $\mu\text{g}/\text{day}$ were established as the maximum safe dietary dose with the no observed adverse effect. Symptoms of Se toxicity were assessed by patient interview with questions regarding breath, hair and nail changes. The low adverse effect of Se supplementation was calculated to be 1540–1600 $\mu\text{g}/\text{day}$. An intake of 3200–5000 $\mu\text{g}/\text{day}$ resulted in definite occurrence of selenosis (Reid et al., 2004). On the other hand a level of about 40 $\mu\text{g}/\text{day}$ was suggested as the minimum requirement, while an intake of <11 $\mu\text{g}/\text{day}$ results in deficiency problems (Whanger, 2004).

Se enters the food chain through plants, which is taken up from the soil. The geographic distribution of Se varies from high concentrations in the soils in certain regions of the former USSR, Venezuela and the USA to rather low Se levels in soils in New Zealand, certain regions in China, East Siberia, Korea, and to some extent also the

soils in many parts of Europe (Brtková and Brtko, 1996; Rayman, 2000, 2005; Ferguson et al., 2004).

Human Se-deficiency diseases have been first identified in some regions of China; Keshan disease, an endemic cardiomyopathy, and Kashin-Beck disease, a deforming arthritis (Rayman, 2000). Numerous other studies suggest that Se deficiency is accompanied by loss of some immunocompetency; with both cell-mediated immunity and humoral immunity being impaired (Spallholz et al., 1990). Se deficiency is also linked to the occurrence, virulence, and disease progression of some viral infections (e.g. HIV progression to AIDS). Low serum Se in women increases the risk of miscarriages (Barrington et al., 1996) and in men it is connected with a decrease in sperm motility and chances of fertilization (Scott et al., 1998). The turnover rate of some neurotransmitters is also altered by Se deficiency. Low plasma Se concentrations in the elderly are significantly associated with senility, Alzheimer's disease and depression (Hawkes and Hornbostel, 1996). Se has a role in the thyroid hormone metabolism being part of the deiodinase enzyme (Brtková and Brtko, 1996). The findings have been equivocal regarding a correlation between Se and cardiovascular disease risk (Virtamo et al., 1985; Kardinaal et al., 1997). The other disorders, such as rheumatoid arthritis, pancreatitis and asthma associated

with increased oxidative stress or inflammation might be expected to be influenced by Se levels (McCloy, 1998; Knekt et al., 2000). General oxidative stress response is totally influenced by dietary Se and its enzyme levels (Rayman, 2000).

2.1. Chemical forms of Se and their metabolism

Se exists in mostly organic forms in normal diets. Organic Se is present in foods mainly in the form of selenomethionine, selenocysteine and Se-methylselenocysteine, whereas inorganic Se either as selenite or selenate occurs much less frequently and in very low amounts. Of the organic forms, selenomethionine is the predominant form in most Se rich diets. Both organic and inorganic forms of Se appear to be utilized with similar efficacy in the body to produce selenoproteins (Shiobara et al., 1998) but the Se enters at different points in metabolism depending on chemical form. A metabolic scheme showing Se metabolism is presented in Fig. 1.

Inorganic forms of Se, selenite and selenate are reduced (from the valence +4 and +6, respectively) by glutathione (GSH). A number of intermediate metabolic steps lead to the generation of H₂Se, or they directly enter the metabolic pool (Foster et al., 1986; Lu et al.,

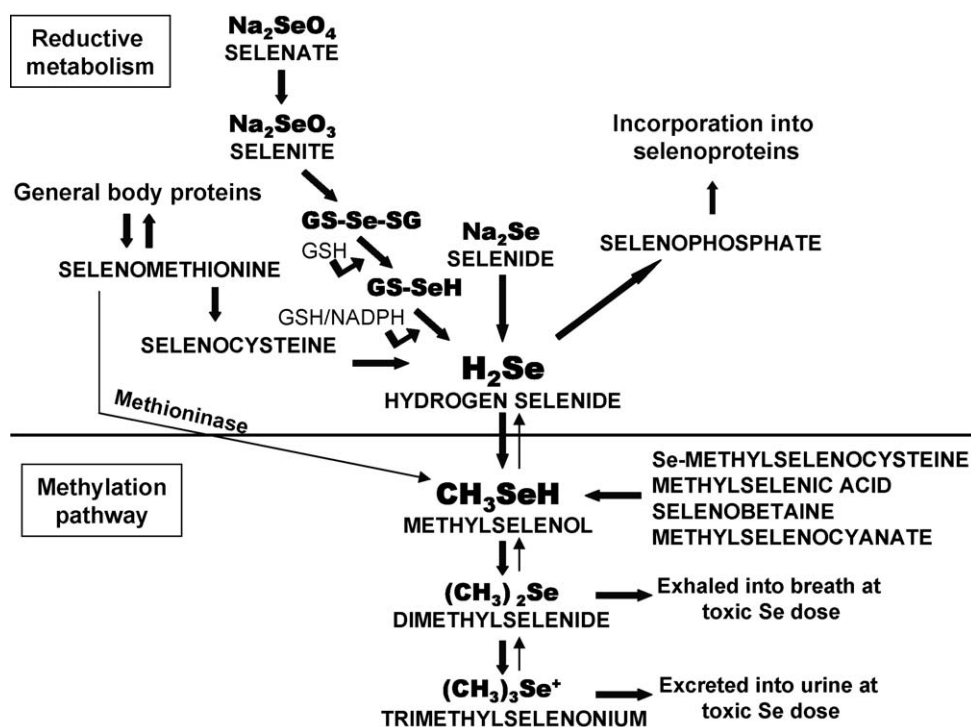


Fig. 1. Schematic representation of the Se metabolic pathway (adapted from Meuillet et al. (2004) and Lu et al. (1995)).

1995). SSe produces H_2Se and/or elemental Se (Se^0) via selenodiglutathione (GSSeSG) through reduction by thiols and NADPH-dependent reductases (Ganther, 1971; Hsieh and Ganther, 1975). This reduction pathway is tightly connected to the production of the superoxide ($O_2^{\bullet-}$) radical (for details, see Section 5).

Organic Se, present in the Se-containing amino acids in foods, is metabolized to the same key intermediate (Esaki et al., 1982; Tanaka et al., 1985). Moreover, H_2Se is the intermediate compound between the reductive metabolism of Se and its methylation pathway. H_2Se either serves as a precursor for the synthesis of selenoproteins, such as glutathione peroxidase, thioredoxin reductase, iodothyronine deiodinases and selenoprotein P, or it undergoes stepwise methylation with the enzymatic reaction of thiol S-methyltransferases to generate the mono-, di-, and tri-methylated forms of Se; methylselenol, dimethyldiselenide and the trimethylselenonium ion, respectively (Ip et al., 1991; Meuillet et al., 2004).

Selenomethionine, the major dietary form of Se, is subject to several different metabolic fates. Firstly, cells do not distinguish between methionine and selenomethionine during protein synthesis, so this natural selenoamino acid gets incorporated into the general body proteins, e.g. albumin, in place of methionine when methionine is or is not a limiting factor. This non-specific incorporation of Se into proteins likely accounts for the observed dose-dependent increase of tissue Se levels with selenomethionine supplemented diets compared with diets supplemented with other chemical forms of Se (Shiobara et al., 1998). Secondly, selenomethionine gets converted into selenocysteine by the trans-sulfuration pathway, which is subsequently converted into H_2Se (Esaki et al., 1981) and follows the same metabolic fate of selenocysteine as described above. Lastly, selenomethionine may generate methylselenol by the enzymatic reaction of methionine α,γ -lyase (also known as methioninase) (Meuillet et al., 2004).

Selenocysteine, another form of dietary organic Se, either taken from diet or derived from selenomethionine, is also converted into H_2Se . Selenocysteine is also synthesized from H_2Se after H_2Se conversion to selenophosphate by selenophosphate synthetase. The insertion of selenocysteine into protein is specified by the UGA codon in mRNA. Thus, selenocysteine forms the active catalytic site in all selenoenzymes and is essential for the synthesis of selenoproteins (Hatfield and Gladyshev, 2002).

Se-methylselenocysteine, unlike selenomethionine, is not incorporated into proteins but may be converted directly to methylselenol by β -lyase (Foster et al., 1986). Like Se-methylselenocysteine, synthetic Se com-

pounds such as selenobetaine, methylseleninic acid and methylselenocyanate also readily generate methylselenol, which is now believed to be the main intermediate metabolite in the cellular events of Se chemoprevention (Combs and Gray, 1998; Ip et al., 2000; El-Bayoumy and Sinha, 2004).

Se compounds that enter either the H_2Se pool or the methylselenol pool undergo methylation by thiol S-methyltransferases and generate different methylated metabolic Se forms that sequentially are exhaled in the breath or excreted in urine contributing to the Se homeostasis of the body. At low Se doses monomethylated forms of Se are excreted into urine as the major form, while trimethylated forms are being predominant at high doses. When the level of trimethylselenonium ion reaches the metabolic plateau, dimethylselenide is exhaled into breath (Itoh and Suzuki, 1997). Selenosugars in urine have recently been identified (Kobayashi et al., 2002) and 1 β -methylseleno-N-acetyl-D-galactosamine is thought to be a major monomethylated urinary metabolite except at extremely high Se intake. Excretion of different Se species in urine at different levels of Se intake may thus be useful indicators of healthy and toxic doses of Se (Kobayashi et al., 2002). Generally, the methylation pathway is considered to be the detoxification pathway for all Se in the diet or in supplements (Foster et al., 1986; Lu et al., 1995).

3. Role of Se in cancer prevention

A low dietary Se intake was first noted in association with cancer risk approximately 35 years ago (Shamberger and Frost, 1969). Human epidemiological studies conducted over this period of time examined the relationship between dietary intake of Se and total cancer risk, and have been somewhat controversial (Meuillet et al., 2004). Early epidemiological studies (Schrauzer et al., 1977a,b) showed a geographic correlation between low Se status and a high incidence of certain types of cancers. Dietary intake of Se in 27 countries found a significant inverse correlation with age-adjusted mortality for cancer of the colon, prostate, breast, ovary and lung as well as with hematopoietic cancers, while only a weak correlation was observed for cancers of the pancreas, skin and bladder. Such reports have been made by more than 100 experimental studies using different forms and doses of Se (Combs and Gray, 1998). Although not all studies showed a correlative effect of high plasma Se level and low cancer incidence (contradictory results are thought to be partially related to the variations in data collection and methods measuring the Se levels), the epidemiological data stimulated interest in testing Se as a

chemopreventive agent in human clinical trials (Meuillet et al., 2004).

Chemoprevention trials have been carried out in China using Se as a single experimental agent. In one study, 130,000 people from the regions with a high incidence of hepatocellular carcinoma (HCC) were recruited. People had their table salt fortified with Se in the form of SSe (15 mg/kg); the other had their salt unfortified. After 6 years, the incidence of HCC decreased by 35% in the Se salt supplemented group in comparison with the unsupplemented control group (Yu et al., 1997).

In another study the effect of low Se concentrations in serum on the incidence of different cancers and on total death were followed. Significant inverse association between base-line serum Se and death from esophageal and gastric (Wei et al., 2004) as well as lung cancer (Zhuo et al., 2004) have been found. These results suggest that Se supplementation may have some protective effects against some type(s) of cancer in populations where average dietary Se levels are low.

The Nutritional Prevention of Cancer Trial carried out by Clark and co-workers in the USA was the first double-blind, placebo-controlled intervention trial in a western population. The trial was designed to test the hypothesis that Se supplementation could reduce the risk of skin cancer (Clark et al., 1996). One thousand three hundred and twelve individuals with a history of non-melanoma skin cancer were randomized to placebo or 200 µg Se per day as selenium yeast. There was no effect on the primary endpoint of non-melanoma skin cancer, however, individuals in the Se-treated group experienced sizable reductions in the risk of primary cancer of colon, rectum, prostate and lung. The prostate cancer incidence decreased by a striking 65% (Clark and Marshall, 2001; Combs et al., 2001; Duffield-Lillico et al., 2002). These findings led to additional clinical studies testing the effects of Se on prevention of primary and secondary prostate cancer (Nelson et al., 2002; Brooks et al., 2001; Van den Brandt et al., 2003). Newer epidemiological data supported the hypothesis that there is a significant inverse correlation between total serum Se level and prostate cancer risk (Vogt et al., 2003; Meuillet et al., 2004; Waters et al., 2005; Etmnan et al., 2005).

Clinical cancer prevention trials demonstrate the potential strategy for reducing the burden of cancer on society. They suggest that nutritional food components, such as Se, when supplemented to diets may minimize or reduce cancer risk. The role of Se in human health is still a subject of intense interest. Large intervention trials with different forms of Se are under way in Europe and the USA to assess the effects of Se supplements in the inci-

dence of cancer and other diseases (Luty-Frackiewicz, 2005; Burk et al., 2006; Sabichi et al., 2006; Drake, 2006).

4. Various mechanisms of Se in cancer prevention

4.1. The protective role of Se in carcinogenesis

It is generally accepted that carcinogen-induced genetic damage via the formation of covalent DNA adducts is necessary, but not sufficient, for the initiation of carcinogenesis. Therefore, several *in vitro* studies in rodents were conducted to examine the effects of various levels and forms of Se on carcinogen DNA adduct formation. In most of these studies, Se (as selenite, selenate, 1,4-phenylenebis(methylene)selenocyanate (*p*-XSC) or diallyl selenide (DASE)) have been shown to inhibit the initiation phase of carcinogenesis induced by various carcinogens in mammary, liver, colon and lung tissue in rats (El-Bayoumy, 2001). In experiments with Se-enriched garlic, Ip and Lisk (1994) reported an inhibition of both the initiation and post-initiation stages of mammary carcinogenesis after dimethylbenz(*a*)anthracene (DMBA) treatment of rats. The explanation for cancer prevention is that some Se compounds affect procarcinogen activation and metabolism through inhibition of phase I and induction of phase II enzymes. Phase I enzymes are members of the cytochrome P450 system responsible for converting chemical carcinogens to reactive adducts that can attack to DNA. On the other hand, defense against carcinogenic injury is provided by phase II detoxifying enzymes. The experiments by Ip and Lisk (1997) showed that increased detoxification of carcinogen via the phase II conjugating enzymes might represent the mechanism of tumor suppression by Se-enriched garlic. This mechanism has been well documented to be important for the chemopreventive activity of many thiol-reactive chemopreventive agents, mainly by inhibition of the initiation stage of carcinogenesis (Zhou et al., 2003).

However, suppression of carcinogenesis by Se compounds in the post-initiation stages of carcinogen treatment in animal models suggests the existence of additional mechanisms for cancer chemoprevention. The ability of Se compounds to inhibit cell growth and to induce tumor cell apoptosis has now been widely demonstrated and is suggested to be a potential mechanism for cancer chemoprevention (Stewart et al., 1997; Wang et al., 2003; Sinha and El-Bayoumy, 2004). It has been speculated that Se-induced apoptosis can remove carcinogen-initiated transformed cells and suppress the

clonal expansion of such transformed cell population (Combs, 1997; Zhou et al., 2003).

Many experiments have shown that the chemopreventive effect of Se is due in part to its inhibitory effect on cell growth, DNA, RNA and protein synthesis in transformed cells (El-Bayoumy, 2001). Although the molecular mechanisms of the cancer chemopreventive activity of the Se compounds remains largely unknown, changes in stress-related cellular proteins have been implicated in explaining the protective effects of Se. Because protein kinases play a central role in the regulation of cell growth, tumor promotion and differentiation, several reports have described the inhibitory effect of Se on kinase activity (Gopalakrishna et al., 1997; Sinha et al., 1999). Moreover, cell cycle CDK2 or cell signaling protein kinases and/or a number of redox-regulated proteins, including the critical transcription factors, such as AP-1 and NF- κ B, have been proposed as targets against which Se exerts its chemopreventive effects (Zhu et al., 1995; Sinha et al., 1996).

4.2. Antioxidant activities of Se

All living aerobic organisms have developed certain defense mechanisms that provide protection against oxidative damage induced by ROS (Valko et al., 2004). The best known of these antioxidant enzymes is superoxide dismutase (Davies, 1994). Se was found to be a component of antioxidant defenses either as agent able to scavenge free radicals, or as a component of a family selenoenzymes. Experimental evidence suggesting its effect in eliminating oxidative stress came in 1973 when Se was found to be incorporated into the first identified selenoprotein, glutathione peroxidase (GPx) (Rotruck et al., 1973). Members of the GPx family of enzymes are effective in catalyzing the reduction of hydrogen peroxide (H_2O_2) and lipid peroxides. In view of the role of GPx in reducing oxidative stress it was proposed that Se-dependent GPx provided a plausible mechanism for cancer prevention. However, it was found that GPx activity was maximized in tissues of animals fed normal amounts of Se and was not elevated as dietary Se was increased 10-fold at higher levels necessary to achieve chemoprevention. Thus, it seemed that Se supplements exerted their chemopreventive effect in a manner unrelated to the saturated levels of GPx (Combs and Gray, 1998). Se is found to be a component of several other selenoproteins, some of which have important enzymatic functions associated with antioxidant defenses. One of these, thioredoxin reductase is considered to be a key enzyme in Se metabolism, reducing Se compounds and controlling the intracellular redox state (Lee et al., 2000;

Madeja et al., 2005). Additionally, iodothyronine deiodinase, produces the active thyroid hormone, T3 from an inactive precursor T4. Another selenoprotein, selenoprotein P appears to protect endothelial cells against damage from free radicals (Allan et al., 1999; Ganther, 1999; Rayman, 2000). Each of these selenoproteins contains Se in the form of selenocysteine, which is incorporated by the co-translational modification of transfer RNA-bound serine at certain loci encoded by a specific UGA codon. As noted above, it has generally been thought that the selenoproteins fulfill only the nutritional requirement for dietary Se. There was not unambiguous evidence supporting the role of any known selenoprotein in cancer prevention. However, it has recently become clear that optimal expression of some selenoproteins, notably selenoprotein P, requires higher amount of dietary Se (Xia et al., 2005) and that a substantial number of individuals may have a higher Se requirement for efficient synthesis of selenoproteins (Rayman, 2005). This inter-individual variation in selenoprotein expression levels may be accounted for single-nucleotide polymorphisms in selenoproteins genes that determine the efficiency with which individuals can incorporate Se into selenoproteins (Kumaraswamy et al., 2000; Ichimura et al., 2004; Apostolou et al., 2004).

Whether the selenoproteins are crucial to the anti-cancer effects needs to be elucidated. The functions of many of the 25 human selenoproteins are as yet unknown, although some of them are involved in antioxidant and anabolic processes (Hatfield and Gladyshev, 2002). Selenoproteins that may be relevant to cancer risk are supposed to be the GPx family, the 15 kDa selenoprotein (Sep15), selenoprotein P and the thioredoxin reductases, although a beneficial role of thioredoxin reductase in cancer prevention is doubtful (Rayman, 2005).

5. Prooxidant toxicity of Se

The effect of Se upon cells is strictly form and concentration-dependent. Se compounds induce the expression of the 25 known selenoproteins, including those with antioxidant activities (Gladyshev and Hatfield, 1999). Many studies have indicated that the cancer chemopreventive activity of Se compounds requires much higher concentration than that required for the synthesis of the selenoproteins (Rayman, 2005). At moderate, supranutritional doses, Se compounds inhibit cell growth and have a prooxidant activity, generating superoxide. At higher concentrations of mainly inorganic forms of Se compounds, acute toxicity due to DNA strand breaks occur (Combs and Gray, 1998). Which of these effects elicited by the supranutritional and/or toxic

Se doses are involved in chemoprevention and tumor cell growth inhibition remains to be elucidated. Several suggested mechanisms such as effect of Se upon oxidative stress, inhibition of DNA synthesis, effect on DNA repair, induction of apoptosis, induction of certain Se metabolites, effect on immune system and inhibition of angiogenesis have been postulated (Whanger, 2004). It is believed that the most plausible explanation for these Se-associated events, such as the carcinostatic activity of some but not all Se compounds, is a requirement for methylselenol formation for carcinostasis and induction of cell death. This hypothesis is based on the ability of all selenides (RSe^-) to generate some degree of oxidative stress in target cells (Spallholz, 1997). Exposure of cells to free radicals damages structure and consequently interferes with the functions of enzymes, nucleic acids and critical macromolecules. The prooxidant activity of Se compounds seems to have both harmful and beneficial role (Stewart et al., 1999). Oxidative stress is supposed to be a major mechanism of Se-induced cytotoxicity and it is involved in the ability of Se compounds to induce apoptosis (Kim et al., 2004; Drake, 2006).

In 1941, Painter was first to propose that Se toxicity was due to its interaction with thiols (Painter, 1941) and these Se reactions were later investigated by others (Ganther, 1968, 1971). It was confirmed that SSe is an effective catalyst for the oxidation of GSH forming selenodiglutathione (GSSeSG). Moreover, it was proposed that Se toxicity was due to the interaction of Se with the disulfides and thiol groups of proteins forming selenotrisulfides (RSSeSR), similar to that shown for GSSeSG, with resultant inhibition of enzyme activity. Selenotrisulfides are relatively stable (Spallholz, 1994), however, they can be reduced by excess thiols or by cellular glutathione reductase, forming a highly reactive selenopersulfide anion ($RSSe^-$).

The first direct evidence of free radical generation by Se compounds was published in 1989 by Seko et al. (1989) who showed that the oxidation of GSH by SSe leads to the production a free radical $O_2^{\bullet-}$, and elemental Se^0 with the intermediate formation of GSSeSG (Fig. 2). Although $O_2^{\bullet-}$ has relatively low reactivity and toxicity, it gives rise to secondary oxidative products. The dismutation of $O_2^{\bullet-}$ yields H_2O_2 , which on its own is not

toxic, but its high reactivity is realized *in vivo* through metal-catalyzed Fenton, or Fenton-like reactions, where H_2O_2 reacts with partially reduced metal ions such as Fe^{2+} or Cu^+ to form $\bullet OH$. In contrast to H_2O_2 , $\bullet OH$ can directly inflict DNA damage and it is considered the most important radical in ROS-related DNA damage (Sigler et al., 1999; Valko et al., 2004).

There is a great deal of chemical and biological evidence that SSe is a prooxidant catalyst (Spallholz, 1994, 1997). Similarly to sulfur, Se can exist in various oxidation states, specifically in association with three oxygen atoms (selenite, SeO_3 , Se^{4+}), or four oxygen atoms (selenate, SeO_4 , Se^{6+}). Analogous to sulfur, Se in organic forms is bivalent (e.g. selenocysteine or selenomethionine). As shown in Fig. 1, the metabolism of Se shows a complex pathway of conversion of inorganic into organic forms producing metabolites with important biological functions. For example, it has been suggested that Se compounds that prevent cancer must continuously produce methylselenol (CH_3SeH) (Ip et al., 2000). Moreover, it has been postulated (Spallholz, 1997; Spallholz et al., 2004) that the anticarcinogenic action as well as toxicity of Se compounds is due to the catalytic nature of the selenide anion (RSe^-), the subsequent generation of $O_2^{\bullet-}$ and oxidative stress, which induces apoptosis and may contribute to carcinostatic activity.

Many compounds of different Se chemical composition have been used for dietary as well as cellular experimental purposes. Of them, inorganic SSe was the agent of choice used either as a supplement to animal diets or as a model in many basic Se biological and molecular research experiments. It was confirmed that SSe generates ROS in the presence of clinical concentrations of sulfhydryl compounds, such as cysteine or GSH and caused cellular damage in human cells (Terada et al., 1999). Due to SSe toxicity, attention has also been directed to a variety of natural and manmade organic Se compounds, many of them potentially pharmacologically important. Research has mostly been focused on the two dietary selenoamino acids, L-selenomethionine and L-Se-methylselenocysteine. Spallholz et al. (2004) have systematically cataloged many Se compounds that can or cannot generate $O_2^{\bullet-}$ using an *in vitro* chemiluminescent assay. They showed that Se compounds that

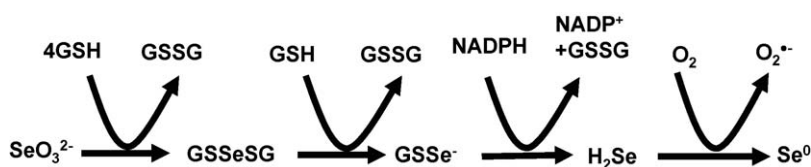


Fig. 2. Selenite reduction pathway and generation of superoxide (adapted from Spallholz (1997), originally published by Seko et al. (1989)).

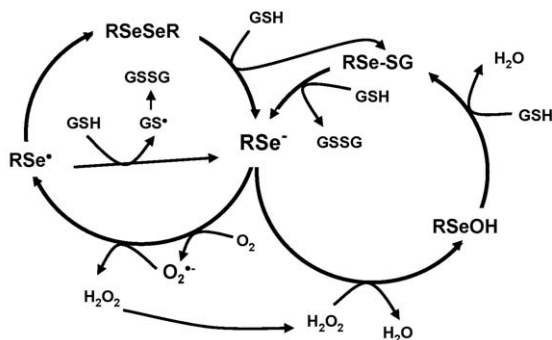


Fig. 3. Redox cycling of selenides (RSe^-) (adapted from Spallholz et al. (2004), originally published by Chaudiere et al. (1992)).

can easily form the selenide anion, RSe^- , also generate $\text{O}_2^{\bullet-}$ *in vitro* via oxidation of GSH, or other thiols (RSH). The two selenoamino acids, L-selenomethionine and L-Se-methylselenocysteine, do not redox cycle *in vitro* because they can not directly form methylselenol via GSH reduction. The methylselenide anion (CH_3Se^-) must be formed from these selenoamino acids *in vivo* by metabolic enzymes, methioninase (in the case of L-methionine) or β -lyases (in the case of L-Se-methylselenocysteine) (Fig. 2). Methylselenol can be directly formed from methylseleninic acid *in vitro* by glutathione reduction and it is a potent redox Se species (Fig. 3).

6. Se and DNA damage

It has been known for a long time that some Se compounds have the potential to induce DNA damage. Much of what is known about the DNA damage by Se is derived from bacterial as well as cell culture experiments. In a majority of DNA damaging experiments, SSe has been chosen as the source of Se. It is now well established that the toxicity of the SSe is strongly influenced by its metabolism and production of ROS (Fig. 1, see Section 5). In accordance, it was shown (Kramer and Ames, 1988) that reactions of SSe with thiols and the concomitant production of ROS were the primary causal events of Se mutagenicity and toxicity in *Salmonella typhimurium*. Studies of isolated hepatocytes as a model system indicated that SSe-induced DNA fragmentation was oxygen and redox cycle-dependent (Garberg et al., 1988), thereby confirming the view that DNA damage by SSe in mammalian cells is also mediated by ROS. Later, it was found that SSe at concentrations of 5–10 μM induced DNA single-strand breaks (SSB) and double-strand breaks (DSB) in murine leukemic L1210 cells and other murine mammary carcinoma cell lines. Induc-

tion of DNA strand breaks was in all experimental cases associated with a loss of cell viability (Lu et al., 1994, 1995). SSe has also led to chromosomal damage in Swiss albino mice and human peripheral lymphocytes (Biswas et al., 1997, 2000). These data clearly indicated that DNA damage is implicated in the genotoxicity of SSe and that SSe-induced toxicity is mediated by its prooxidant activity connected with ROS formation.

The organic Se compounds, selenomethionine and selenocysteine, using the different metabolic steps produce the same metabolite H_2Se , as SSe (Fig. 1). The production of the toxic H_2Se would suggest that both inorganic and some natural organic forms of Se can be effective in induction of DNA damage, although not necessarily with similar dose efficiency. Accordingly, DNA damaging activity of selenomethionine have recently been demonstrated in prostate cells of dogs fed by high doses (6 $\mu\text{g}/\text{kg}/\text{day}$ for 7 months) of this dietary amino acid (Waters et al., 2005). When organic methylated Se compounds were examined for potency to induce DNA strand breaks, a distinctive cellular response in comparison with SSe was reported (Ip, 1998). In cell culture, SSe at levels of 5–10 μM can induce SSB, cell death by necrosis and acute cell lysis. In contrast, the methylated Se compounds, such as methylselenocyanate or methylselenocysteine, even at levels of 10–50 μM induced cell death predominantly by apoptosis, an event that is characterized by specific morphological (condensation of chromatin) and biochemical (DNA fragmentation) changes without evidence of DNA strand breakage (Wilson et al., 1992; Lu et al., 1995). Differences in the DNA damaging activities and in the induction of morphological changes by SSe versus methylselenocyanate indicated induction of different metabolic pathways by these two agents leading predominantly to H_2Se by SSe and to methylselenol by methylselenocyanate. Compounds that are directly converted to methylselenol, such as methylselenocyanate (Fig. 1), are supposed to be superior to the inorganic SSe as well as to the organic selenomethionine due to their significant preventive efficacy and the minimal side effects on DNA stability and toxicity (Combs and Gray, 1998; El-Bayoumy and Sinha, 2004; Whanger, 2004).

Previous studies have suggested that DNA damage is involved in the induction of events leading to apoptotic death in tumor cells (Lu et al., 1994). Recently, it has been demonstrated that SSe-induced apoptosis, which involved ATM/ATR and TOP II, is likely to be caused by DNA damage (Zhou et al., 2003). Thus, DNA damage seems to play an important regulatory role in SSe-induced apoptosis.

7. Se and DNA repair

Among the essential trace mineral nutrients, Se has a special position due to its catalytic activity and toxicity. In comparison to our understanding of the protective role(s) of Se in human health and cancer prevention, less information is available about the mechanisms which are responsible for maintaining of genetic information and for repairing DNA lesions induced by Se's prooxidant character. As mentioned above, SSe is an inorganic oxidizing agent, which has the capacity to compromise genetic stability by inducing DNA damage. Although the exact mechanism of SSe-induced modifications to DNA remains unknown, DNA strand breaks (Lu et al., 1995; Zhou et al., 2003) and base lesions (Wycherly et al., 2004) seem to be the most probable consequence of SSe-induced toxicity.

Data accumulated over many years clearly shows that oxidative DNA damage plays an important role in a number of disease processes, including malignant transformations (Brozmanová et al., 2001; Evans et al., 2004). Because there is a relatively narrow range separating the amount of Se essential for health from that which is toxic, it is necessary to estimate the possible genetic risk of Se compounds against their beneficial effects to the humans. This information is also important to evaluate the safety of possible pharmacological application of Se-containing agents such as the anticancer drugs.

To reduce the adverse consequences of many environmental agents, including Se, a complex network of different repair systems has evolved to maintain genetic integrity (Friedberg, 2000; Hoeijmakers, 2001). The major pathway eliminating DNA base damage, base free sites and SSB is excision repair, subdivided into nucleotide excision repair (NER) and base excision repair (BER). The replication errors are repaired by the mismatch repair (MMR) pathway and DSB by homologous recombination (HR) or non-homologous end-joining (NHEJ).

Nevertheless, besides agents that induce exclusively different types of DNA damage, either directly or indirectly via intermediates, there are some agents which can also interfere with the repair of DNA thus increasing the adverse consequences of DNA lesions. Well recognized examples are the carcinogenic metal compounds, such as nickel, cobalt, cadmium and arsenic which were shown to inhibit BER and NER repair pathways at low, seemingly non-cytotoxic concentrations. Potential target molecules for metal ions are the zinc finger structures in DNA repair proteins, with each zinc finger protein sensitive towards a specific toxic metal ions (Hartwig et al., 2002; Hartwig and Schwerdtle, 2002). Moreover,

it was shown that zinc fingers may be sensitive targets not only for non-essential toxic metal ions, but also for essential trace elements such as Se. Thus, it was demonstrated *in vitro* that certain reducible Se compounds caused a concentration-dependent decrease of activity and released of zinc from the zinc finger motif in the two zinc finger repair proteins, bacterial formamidopyrimidine DNA glycosylase (Fpg) involved in BER and xeroderma pigmentosum group A protein (XPA) essential for NER (Hartwig et al., 2003; Blessing et al., 2004). These results demonstrate that at low concentrations Se compounds that are reducible to selenides may inactivate the DNA repair processes by the oxidation of zinc fingers in DNA repair proteins (Ho, 2004). Moreover, it has been shown that treatment of human lymphocytes and osteosarcoma cells with SSe in the range 0.01–10 μM led to a decrease of the ability to repair radiation induced chromosome deletions and chromatid breaks and to reactivate the cisplatin-treated reporter system, respectively (Abul-Hassan et al., 2004). These observations suggest that Se might have a direct inhibitory effect on DSB repair and transcription-coupled repair (TCR) processes. In opposition to these findings, there are observations that selenomethionine increases the DNA repair capacity in human fibroblast cells damaged by UV light and H_2O_2 (Seo et al., 2002) and that SSe and selenomethionine protect keratinocytes from UV-induced oxidative DNA damage (Rafferty et al., 2003). Such contradictory data might be a consequence of the complexity and diversity of events induced by the different forms and doses of Se compounds. Moreover, it appears that Se status has multilayered effects on both DNA integrity involving antioxidative defenses, oxidative stress and signaling pathways, and on the integrity and function of DNA repair proteins involving oxidation of SH groups and zinc release from zinc fingers.

8. The yeast *Saccharomyces cerevisiae* as a possible model to test mechanisms of DNA damage and repair induced by Se

The eukaryotic budding yeast, *S. cerevisiae*, has already proven to be a powerful model system for DNA repair studies on both cellular and molecular levels. DNA repair studies using *S. cerevisiae* led to the discovery of several important repair phenomena and pathways highly relevant to areas of investigation in human biology and diseases (Resnick and Cox, 2000). Systematic research of mutagen-cell interaction in *S. cerevisiae* led to the discovery of a number of genetic loci having functions in DNA repair. Yeast mutants originally isolated by a virtue of their radiation sensitivity have been organized

into three epistasis groups designated *RAD3*, *RAD6* and *RAD52*, each representing a different DNA repair system. Phenotypic characterization of these mutants indicated that the *RAD3* epistasis group comprised genes involved in NER. Mutants in the *RAD52* epistasis group were defective in genetic recombination and DSB repair; and these genes are therefore believed to be required for recombinational DNA repair. Many of the genes in the *RAD6* epistasis group were required for spontaneous and/or damage-induced mutagenesis, suggesting their participation in biochemical events associated with altered replication fidelity. Other genes not designated as *RAD* loci are those encoding proteins involved in the repair of lesions induced by mono- and bi-functional psoralens (*PSO*), BER factors, MMR factors and those operating in DSB repair by NHEJ (Brozmanová et al., 2004).

To establish the molecular basis of SSe toxicity and resistance, the yeast mutants compromised in the individual repair pathways were tested for their sensitivity to SSe (Pinson et al., 2000). Effect of SSe on the doubling time of various DNA repair deficient mutants grown in complete medium showed that the *rad9* mutant significantly reduced the cell growth rate after SSe treatment, suggesting that *RAD9*-dependent mitosis checkpoint is critical for proper repair of SSe-induced DNA damage. Neither the NER gene, *RAD1*, nor oxidative DNA damage repair genes belonging to the BER pathway, *OGG1*, *NTG1*, *NTG2* and *APN1*, play a role in repairing SSe-induced DNA lesions. Therefore, the BER mechanism, which is involved in the repair of oxidative DNA damage, does not seem to be involved in the repair of SSe-induced DNA damage. Interestingly, mutations in the *RAD51* and *RAD52* genes, which encode components of the DSB repair machinery in yeast (Dudáš and Chovanec, 2004), reduced the growth rate after SSe treatment only very moderately, indicating that the major effect of SSe in yeast probably does not involve DSB. In contrast, more recent data have shown that the recombination-deficient *rad52* mutant exhibited extreme sensitivity to diphenyl diselenide (DPDS), a Se compound that was shown to have a prooxidant activity generated by GSH depletion in yeast (Rosa et al., 2004; Moreira et al., 2005). The hypersensitivity of the *rad52* mutant strain to DPDS suggested that HR is critical for the processing of the potentially lethal genetic lesions resulting from DPDS-induced DNA damage. This conclusion is in good accordance with our recent, yet unpublished, data demonstrating that the *S. cerevisiae* mutant strain affected in HR is hypersensitive to SSe and unable to repair DSB induced by this agent (Letavayová et al., in preparation). Thus, the HR pathway seems to be the

main defense mechanism by which cell deals with DNA damage induced by Se compounds.

9. Conclusions

Among dietary trace elements, Se has been found to have special attributes due to its multilayered activities (Combs and Gray, 1998; Whanger, 2004). It is dietary essential, being specifically incorporated into the active sites of several known proteins or enzymes as an amino acid, selenocysteine. It is pharmacologically active and at supranutritional dietary levels can prevent the development of many cancers, thus demonstrating chemoprevention and/or carcinostatic activities (Rayman, 2005). At higher dietary levels, many Se compounds can become toxic (Spallholz, 1994). All these attributes of Se mainly depend upon the concentration, the chemical form and metabolic activity of the compound (El-Bayoumy, 2001; Whanger, 2004). A common specific characteristic of Se compounds that express the carcinostatic activity and toxicity *in vitro* and *in vivo* is their interaction with thiols and the generation of free radical species (Kramer and Ames, 1988; Spallholz, 1997). In accordance with the prooxidant activities of Se, the higher doses of some Se compounds have the potential to induce DNA damage (Lu et al., 1994, 1995; Zhou et al., 2003; Wycherly et al., 2004; Waters et al., 2005). Moreover, due to its reactivity with thiols, Se can also interfere at the same time with integrity and/or function of DNA repair proteins, thereby increasing the adverse consequences of induced DNA lesions (Hartwig et al., 2003; Blessing et al., 2004).

Naturally occurring forms of Se, such as organic selenomethionine and selenocysteine are components of the normal diet. Both organic selenomethionine (as a prominent component of Se-enriched yeast) and inorganic SSe are commercial forms of Se compounds that are available to, and taken as supplements by the public (Shen et al., 2001; Whanger, 2002). Several organoselenium compounds have been shown to have promising cancer preventing activity and some of them are used in the synthesis of pharmacologically active drugs (El-Bayoumy and Sinha, 2004). Because the public may frequently supplement Se compounds at high doses, the possible prooxidant effect of Se requires more attention. Taken together, high Se intake either as a result of dietary or pharmacological supplements could at certain circumstances expose the body tissues to toxic levels of these compounds with subsequent negative consequences on DNA integrity and repair. On the other hand, there is no doubt that Se compounds play very important role in cancer prevention either as components of antioxidant enzymes, or as anticarcinogenic metabolites.

However, despite the substantial progress in our knowledge about the protective role of Se compounds for our health and cancer prevention, the molecular mechanisms of Se cytotoxicity in association with DNA damage and repair is still less defined. To evaluate the safety of both the dietary and pharmacological applications of Se-containing agents, further studies are urgently needed to elucidate effects of Se on DNA damage induction and repair and hence on overall genomic stability. The budding yeast, *S. cerevisiae*, could be very helpful model system in this respect, because of the existence of a large number of mutants specifically sensitive to only one type of DNA damage. The use of such mutants can reveal DNA lesions induced by Se compounds *in vivo* as well as pathways responsible for their repair.

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