Review

Selenium, oxidative stress, and health aspects

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Abstract

Metabolic processes which generate oxidants and antioxidants are governed by genetic disposition as well as environmental factors. Changes in lifestyle, including increased environmental pollution, sun exposure, and dietary habits modify the challenge of the organism by reactive oxygen species. Defense mechanisms are reinforced by increasing dietary intake of antioxidants and micronutrients such as vitamins and selenium (Se). Se deficiency has been recognized to promote some disease states. Epidemiological findings link a lowered Se status to neurodegenerative and cardiovascular diseases as well as to increased cancer risk. While evidence exists to suggest that additional selenocompounds would be beneficial in some health conditions, results from future intervention trials are needed to substantiate the argument for increasing Se intake. Several pieces of the puzzle concerning the molecular mechanisms underlying the reactive oxygen species-triggered disease state and intervention by enzymatic antioxidants have been elucidated. A novel concept of protection of stromal cells against the dominating influence of tumor cells in tumor–stroma interaction by selenocompounds and other antioxidants is presented herein, which may translate into therapeutic strategies in chemoprevention of tumor invasion.

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Abbreviations:  ROS, reactive oxygen species; SECIS, selenocysteine insertion sequence; SeP, selenoprotein P

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Oxidative stress and antioxidant defense systems

Oxidative stress describes a condition occurring when the generation of reactive oxygen species (ROS) in a system exceeds the system’s ability to neutralize and eliminate them (Sies, 1985, 1986; Sies and Cadenas, 1985). The imbalance can result from a lack of antioxidant capacity caused by disturbance in production, distribution, or by an overabundance of ROS from endogenous sources or environmental stressors. If not regulated properly, excess ROS can damage cellular lipids, proteins or DNA, thus inhibiting signal transduction pathways, and, in general, normal cellular function. Because of this, oxidative stress has been implicated in a growing list of human diseases such as cardiovascular and neurodegenerative diseases and cancer as well as in the aging process (Sies, 1991).

ROS are by-products of cellular metabolism, generated primarily by electron leakage of mitochondrial electron carriers and enzymes during oxidative phosphorylation. In addition, other cellular enzymes or enzyme complexes located in or associated with cellular membranes or organelles were described for the production of ROS in phagocytic and nonphagocytic cells (Meier et al., 1989). Representative external sources for ROS are redox cycling xenobiotics (Kappus and Sies, 1981) as well as radiation, e.g. ultraviolet light (Brenneisen et al., 1998). A variety of cytokines and growth factors are known to generate ROS which via alterations of the intracellular redox state and/or oxidative modifications of proteins exert their mechanisms of action on signaling components and transcription factors (Thannickal and Fanburg, 2000).

Antioxidants are natural or synthetic molecules preventing the uncontrolled formation of ROS or inhibiting their reactions with biological structures. Antioxidant defense involves multiple strategies, enzymatic or non-enzymatic (Sies, 1993). Non-enzymatic compounds comprise tocopherols, carotenes, ascorbate, glutathione, ubiquinols, and flavonoids. In addition, there is emerging attention on micronutrient elements such as selenium (Se) and zinc which are important as integral constituents of protective enzymes via special amino acids (e.g. selenocysteine, selenomethionine) or structural components (e.g. Zn fingers, Zn-metallothionein) (Klotz et al., 2003).
Overall, these low-molecular-mass antioxidant molecules add to the enzymatic defense system provided by superoxide dismutases, catalase, thioredoxin reductases, and glutathione peroxidases (Sies, 1997).

2. Selenium utilization and metabolism

Se is an essential trace element, and humans take up Se predominantly from grains, cereals and meat. A complex reaction cascade may convert inorganic Se compounds such as selenate and selenite to organic forms and vice versa, which can be enzymatically catalyzed (Ip, 1998). Hydrogen selenide (H$_2$Se) plays a central role, formed from glutathione-coupled reactions from selenite (SeO$_3^{2-}$) via selenodiglutathione (GS-Se-SG) and glutathione selenopersulfide (GS-SeH). H$_2$Se is generally regarded both as substrate for biosynthesis of selenocysteine (Sec) by cysteine synthases and as molecule for the transformation into selenophosphate (H$_2$SePO$_4^-$) by selenophosphate synthetase, and both are required for the biosynthesis of selenoproteins. Further metabolism of H$_2$Se involves sequential methylation to methylselenol (CH$_3$SeH), dimethylselenide ((CH$_3$)$_2$Se), and trimethylselenonium ion ((CH$_3$)$_3$Se$^+$), the latter both exhaled by breath, or excreted in urine. Alternatively, selenomethionine, which can be incorporated into proteins in place of methionine, converts to selenocysteine through transsulfuration, which in turn is degraded to H$_2$Se by cysteine lyase (Birringer et al., 2002; Ganther, 1999; Ip, 1998).

Se is incorporated into proteins as selenocysteine, designated the 21st amino acid, which is specified in the genetic code by the UGA codon, typically serving as stop codon. Sec incorporation occurs cotranslationally at this UGA codon in prokaryotes and eukaryotes (Berry et al., 2001; Zinoni et al., 1987). In contrast to prokaryotes, eukaryotes need the assembly of a stable insertion complex, consisting of the Sec codon, a special ‘stem loop’ structure called selenocysteine insertion sequence (SECIS) located in the 3’ untranslated region (UTR) at considerable distances from the UGA codon, the Sec-tRNA$^{\text{Sec}}$, the Sec-specific elongation factor (eEF$^\text{Sec}$), and the UTR binding protein SECIS binding protein-2 (SBP2) (Thanbichler and Böck, 2001; Tujejaveja et al., 2000). SECIS elements function by recruiting SBP2 to form a tight SECIS-SBP2 complex and may also be stably associated with ribosomes. Besides binding to SECIS elements and ribosomes, SBP2 binds the eukaryotic elongation factor eEF$^\text{Sec}$, which in turn recruits Sec tRNA$^{\text{Sec}}$ and inserts Sec into nascent polypeptides in response to UGA codons (Hatfield and Gladyshev, 2002).

Twenty-five eukaryotic selenoproteins have been identified (Kryukov et al., 2003), however, between 30 and 50 mammalian selenoproteins are expected to exist, which may be identified in the human genome using bioinformatic approaches (Behne and Kyriakopoulos, 2001). The importance of selenoproteins for life was demonstrated by knock-out mice lacking the Sec-tRNA$^{\text{Sec}}$ gene, which resulted in early embryonic lethality (Bosl et al., 1997). Se-containing proteins known so far are divided into three groups comprising (i) proteins into which Se is incorporated nonspecifically, (ii) proteins specifically binding Se, and (iii) “true” selenoproteins, containing Se in the form of genetically encoded selenocysteine (Behne and Kyriakopoulos,
Selenoproteins with known functions play a critical role in a variety of biological processes, and several of them are involved in antioxidant defense. For example, four glutathione peroxidases, among them cytosolic glutathione peroxidase, the first identified selenoprotein (Flohé et al., 1973), protect cells against peroxidative damage by reducing hydrogen peroxide, free fatty acid hydroperoxides, and phospholipid hydroperoxides (Brigelius-Flohé and Flohé, 2003). The family of deiodinases consists of three members which differ with regard to their tissue distribution and their role in catalyzing the activation and inactivation of thyroid hormones (Behne et al., 1990; Behne and Kyriakopoulos, 2001). In mammals, three distinct mammalian thioredoxin reductases function in cellular redox homeostasis by reducing thioredoxin and other substrates (Tamura and Stadtman, 1996). The function of the majority of selenoproteins is poorly defined or unknown, including selenoprotein W (SelW) which is expressed in cardiac and skeletal muscle (Birringer et al., 2002). As for selenoprotein P (SeP), an antioxidant function (Arteel et al., 1998; Saito et al., 1999) as well as a pivotal role in delivering hepatic Se to target tissues have been proposed (Hill et al., 2003; Schomburg et al., 2003).

3. Reactive oxygen species and selenium: regulation of cell signaling

Current concepts of ROS signaling comprise two general mechanisms of action, namely, alterations in intracellular redox state and oxidative modifications of proteins. The former mechanism is accomplished by thiol redox systems, mainly glutathione and thioredoxin, both counteracting intracellular oxidative stress by reducing \( \text{H}_2\text{O}_2 \) and lipid hydroperoxides (Thannickal and Fanburg, 2000). Furthermore, critical cysteines in redox sensitive transcription factors AP-1 and NF\( _\kappa \)B can be reduced by thioredoxin and redox factor Ref-1, subsequently increasing transcriptional activities of both transcription factors by enhancing their ability to bind DNA (Hirota et al., 1999). The major oxidative modification of critical amino acids within functional protein domains involves the sulfhydryl group of cysteine residues, which may be oxidized to the disulfide and to sulfenic and other derivatives (Jacob et al., 2003). In that context, protein-tyrosine phosphatase 1B was inactivated by ROS-mediated oxidation of a cysteine at its catalytic site, which has been proposed as a mechanism for growth factor triggered signaling (Barrett et al., 1999).

The importance of Se is characterized by its role as a constituent of several key antioxidants as well as the unique redox characteristics of selenocysteine and its use in antioxidant enzymes such as thioredoxin reductase. The reduction of reactive oxygen metabolites by glutathione peroxidases helps to maintain membrane integrity. The proposed catalytic mechanism of selenocysteine-containing enzymes for the reduction of hydroperoxides (ROOH) and peroxynitrite (ONOO\(^-\)) comprises oxidation to the corresponding selenenic acid (R-SeOH), which in turn results in reduction of ROOH and ONOO\(^-\) to the corresponding alcohol and nitrite (Sies et al., 1997). Several low-molecular-weight selenocompounds (e.g. selenomethionine, RSe\( _\mathrm{R} \)) mimic the selenocysteine reaction. The formed selenoxide (RSeOR\( _\mathrm{R} \)) is reduced, for example, by thioredoxin reductase at the expense of NADPH (Klotz 2001).
Inhibitory mechanisms of Se compounds involving modification of cysteine residues in proteins were demonstrated which involve both the formation of Se adducts/intermediates of the selenotrisulfide (S-Se-S) or selenenylsulfide (S-Se) type and oxidation of thiol groups as well as reduction of disulfide bonds (Ganther, 1999). In that context, the activities of extracelluar signal-regulated kinases (ERK) 1 and 2 were stimulated by the presence of selenate in adipocytes and hepatocytes, which seems to be mediated by oxidation of –SH groups of the proteins (Pillay and Makgoba, 1992). Conversely, stimulation of the p38 mitogen-activated protein kinase and protein tyrosine nitrosylation by peroxynitrite is suppressed by selenite supplementation of rat liver epithelial cells (Schieke et al., 1999).

The molecular and mechanistic aspects of Se compounds are still under discussion and often are a ‘double-edged sword’: micromolar levels of selenite and methylselenol facilitate intramolecular S–S bond formation in the cysteine-containing catalytic subunit of protein kinase C leading to enzyme inactivation in precancerous cells. By contrast, thioredoxin reductase reverses Se-induced inactivation of protein kinase C, which explains how resistance to Se may develop in advanced malignant cells (Gopalakrishna and Gundimeda, 2002).

4. Selenium and human health: impact on cancer

In the available literature, it is stated that Se is needed for functioning of the immune system, and also is implicated in delaying the aging process. Se deficiency has been linked to diabetes and a number of disorders such as cardiovascular and neurodegenerative diseases. In that context, delivery of Se to the brain for selenoprotein synthesis and SeP, identified as neuronal survival factor (Yan and Barrett, 1998), are crucial for normal neuronal development (Chen and Berry, 2003). Serum, plasma, and urine Se levels are lowered in a variety of cancer types compared to both matched and unmatched controls, and the majority of epidemiological studies provide evidence for Se as a chemopreventive compound for specific cancers. The anticarcinogenic effectiveness of Se depends on its chemical form and dosage (Ip, 1998). In a prospective, placebo-controlled, double-blind study with participants with a history of non-melanoma skin cancer designed to test the hypothesis that Se supplementation could reduce the risk of cancer, Clark et al. (1996) observed that Se intake of 200 μg/day in the form of enriched yeast, containing several Se metabolites, resulted in lower total cancer mortality and significantly lower incidence of various types of secondary cancer (e.g. lung, prostate, colon) compared to placebo controls; however, there was no effect on the recurrence of melanoma and non-melanoma skin cancer. Interestingly, analysis of the treatment effect by measurements of the baseline plasma Se status indicated that the strongest protective effect was seen in individuals with the lowest initial Se level (<106 ng/ml) (Combs, 2001). Furthermore, preclinical, epidemiological, and phase III data from randomized, placebo-controlled clinical trials of the Se and Vitamin E Cancer Prevention Trial (SELECT) suggest that both Se and vitamin E have potential efficacy in prevention of prostate cancer (Klein et al., 2003). In addition, selenite diminished
the expression of distinct members of the matrix metalloproteinase family in HT1080 human fibrosarcoma cells, resulting in lowering of the invasive capacity of these tumor cells (Yoon et al., 2001).

The protection from excess ROS involved in cancer development (Cerutti, 1994) involves glutathione peroxidases, thioredoxin reductases and possibly other selenoproteins (e.g. SeP) containing Se in the form of selenocysteine. The effect of Se on modulating the activity of these proteins is one possible means by which Se might

![Graph](image_url)

**Fig. 1.** Modulation of gap junctional communication and novel concept in chemoprevention. (A) The number of Lucifer Yellow-stained collateral cells adjacent to the microinjected cell was used as a measure of GJIC after 1 min. Confluent human dermal fibroblast (HDF) monolayer cultures were incubated with 0.5 µM Na₂SeO₃ for 48 h prior to treatment with supernatant (SN) of SCL-1 tumor cells, harvested after 2 days (SN(2d) SCL-1), or conditioned medium of HDF, harvested after 2 days (SN(2d) HDF), for additional 24 h. The values of the box plots represent the number of communicating cells of 30 microinjected cells (10 cells/dish). Similar results were obtained in two independent experiments. *P > 0.05 versus SN(2d) SCL-1 (Mann–Whitney test). (B) Novel concept of chemoprevention of tumor progression. Tumor cells release growth factors and cytokines, which via receptors initiate ROS-dependent changes of gene expression in stromal cells resulting in proinvasive signals. Proinvasive signals stimulate invasive capacity of cancer cells. These signals are lowered or prevented by treatment of stromal cells with selenocompounds and other antioxidants.
suppress carcinogenesis. Genetic data indicate that functional polymorphisms in the
genes for several selenoproteins, for example cytosolic glutathione peroxidase, as
well as allelic loss of selenoprotein genes promote cancer development (Diwadkar-
Navsariwala and Diamond, 2004). Aside from the antioxidant activity of Se-contain-
ing enzymes, other reactive forms of Se participate in a variety of reactions, relevant
to its anticarcinogenic effect. Several metabolite pools seem to be of particular
importance, namely selenite, selenodiglutathione, methylselenol, selenomethionine,
and Se-methylselenocysteine (CH$_3$SeCH$_2$$_2$(NH)$_2$COOH) (Ip, 1998), whose molecular
action deals with alterations in the metabolism of endo- or exogenous carcinogens
(El Bayoumy, 2001) as well as redox changes in (tumor) cells leading to inhibition
of proliferation and enhancement of apoptosis (Fleming et al., 2001). However,
the exact mechanisms of the anticarcinogenic effect of Se remain open. In this regard,
the prooxidant effects of Se at higher dosages was also suggested to play a role in
some of the anticarcinogenic properties of Se (Spallholz, 1994).

 Recently, Stuhlmann et al. (2003, 2004) reported that tumor cell-triggered and
ROS-dependent downregulation of gap junctional intercellular communication be-
tween stromal fibroblasts, a hallmark in carcinogenesis, was counteracted by pretreat-
ment of the stromal cells with low, non-toxic concentrations of selenite. Interestingly,
the protective effect of selenite is abrogated by preincubation of the fibroblasts with
buthionine sulfoximine (BSO), an inhibitor of $\gamma$-glutamylcysteine synthetase, widely
used to deplete cellular glutathione levels (Fig. 1A). This novel concept of protection
of stromal cells against the dominating influence of tumor cells in tumor–stroma inter-
action by antioxidants or micronutrients reflects an initial approach for new therapeu-
tic strategies in chemoprevention of tumor invasion (Fig. 1B). By cytokines and growth
factors, tumor cells increase the ROS level in stromal cells, which initiates intracellular
signaling and changes in gene regulation, ultimately resulting in proinvasive signals
promoting tumor progression. The use of antioxidants (e.g. selenocompounds, seleno-
proteins) may prevent these changes in stromal cells, which results in lowering of the
invasive capacity of tumor cells.

5. Selenium protein P: novel aspects

 Low levels of SeP (SeP) were shown to be associated with significantly increased
cancer risk (Persson-Moschos et al., 2000). SeP is unique among selenoproteins, contain-
ting two SECIS elements and 10 UGA codons in its mRNA, predicting up to 10
Sec residues in the polypeptide chain. SeP revealed an apparent molecular size of
67 kDa, which is due to glycosylation, though a mass of 41 kDa is suggested from
human SeP cDNA (Aksesson et al., 1994; Burk and Hill, 1994). In addition, multiple
forms of rat and human SeP are present in plasma, as UGA in the C-terminus may
register as stop codons (Dreher et al., 1997). The deduced amino acid sequence of
mouse SeP appears less related to the known SeP sequences of rat and human (Ste-
inert et al., 1997).

 The biological role of SeP has yet to be fully elucidated, but currently there is evidence
for more than one function (Burk et al., 2003; Saito et al., 2004). As SeP is the
major selenoprotein in human blood plasma, it has been postulated to transport Se from liver to various other organs including brain, which was recently supported by two independent studies using SeP knockout mice (Hill et al., 2003; Schomburg et al., 2003). In addition, there is increasing evidence that SeP acts as a scavenger of free radicals in cellular defense against oxidative injury. In rats, SeP protected from oxidative damage of the liver induced by diquat (Burk et al., 1995). As

Fig. 2. Selenite increases steady-state mRNA levels and release of SeP from hepatoma cell line HepG2. (A) Subconfluent (~80% confluence) HepG2 monolayer cultures were treated with different non-toxic concentrations of Na$_2$SeO$_3$ for 48 h. Total RNA was prepared from tumor cells and subjected to RT-PCR for SeP, SECIS binding protein-2 (SBP2, Sec-specific elongation factor (eEFSec), and the housekeeping gene hypoxanthine-phosphoribosyl-transferase (HPRT). For quantification of the PCR fragments, the gels were scanned by an image-analysis system. Quantitative data were standardized to HPRT and densitometric values represent fold increases over control set at 1.0. The data represent means ±SEM from three independent experiments. (B) Subconfluent HepG2 monolayer cultures on 6 cm-tissue culture dishes were treated as described for (A) 40 µl of 2 ml supernatants were used for Western blot analysis of SeP and subjected to 12% SDS-PAGE. After electroblotting on nitrocellulose membrane, immunodetection was carried out using a polyclonal rabbit antibody raised against human SeP (Mostert et al., 1998) and a horseradish peroxidase-conjugated goat-anti rabbit IgG secondary antibody. SeP was detected by a chemiluminescence system. Coomassie-stained albumin was used as loading control. C, control.
mentioned above, Arteel et al. (1998) reported a peroxynitrite reductase activity for reduced SeP in human plasma, preventing peroxynitrite-mediated oxidation and nitration. In this regard, Traulsen et al. (2004) isolated human low density lipoproteins (LDL) from human blood plasma and investigated a possible protective effect of SeP against its oxidation in a cell-free in vitro system. It was found that addition of purified SeP inhibits oxidation of LDL exposed to copper(II) chloride or to 2,2'-azobis(2-amidinopropane), using the formation of conjugated dienes in LDL as parameter for lipid peroxidation. The protection of LDL from oxidation provides further evidence for the antioxidant capacity of SeP. Because SeP associates with endothelial membranes, it may act in vivo as a protective factor inhibiting the oxidation of LDL by reactive oxygen species. SeP was purified from human blood plasma by three successive chromatography steps (Mostert et al., 1998; Traulsen et al., 2004). Recently, we showed that low, non-toxic concentrations of selenite resulted in a 5-fold increase in SeP steady-state mRNA levels in the human hepatoma cell line HepG₂ compared to untreated controls, whereas SBP2 and eEFSec mRNA levels were only marginally increased (Fig. 2A). Furthermore, this correlated with a significant increased protein amount of SeP in the supernatant of HepG₂ cells (Fig. 2B), which may be purified and used for further studies on antioxidant function of SeP. Two bands of SeP are shown due to differences in glycosylation and/or use of distinct UGA as stop codon.

Results of studies addressing a cancer protective effect of SeP have been controversial so far. Due to its potential as antioxidant, SeP is considered to provide protection against ROS, thereby reducing DNA damage and preventing some types of cancer. A significant lowering or loss of SeP mRNA levels were detected in colorectal cancers compared with corresponding normal mucosa (Al Taie et al., 2004). Conversely, higher expression of SeP in some tumors compromise the efficiency of ROS-inducing chemotherapeutics (Maehara et al., 2004).

6. Concluding remarks

Knowledge on import and management of the trace element Se at the cell biological, biochemical, and molecular biological level has increased considerably in recent years (Sies and Packer, 2002). Recent evidence underlines the importance to health of adequate Se status, but overconsumption by excess use of Se supplements needs to be discouraged because of Se toxicity, with a fairly small therapeutic window. With regard to mechanistic issues, we look forward to a more complete understanding of molecular aspects of Se-dependent chemoprevention. Accumulating knowledge on effects of selenocompounds and selenoproteins will help the development of new therapeutic strategies. In this regard, ebselen, an organoSe compound acting as a glutathione peroxidase mimic, was shown to suppress superoxide anion formation and release of nitric oxide (Wang et al., 1992) as well as to scavenge peroxynitrite and to protect against lipid peroxidation, which fits to its proposed potential to prevent oxidative processes (Parnham and Sies, 2000).
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