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Prooxidant mechanisms of selenium toxicity – a review

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ABSTRACT Selenium is an essential trace element in living organisms as integral part of seleno-enzymes. However, excess amount of selenium is toxic for so-called non-accumulator plants, animals and humans. The toxicity for plants depends on the capacity of synthesis of non-protein amino acids and also their volatilization in the form of dimethylselenide, while in animals on the rate of methylation and its excretion. In vitro studies showed that there are selenium-resistant animal and human cell lines which showed altered selenium uptake. Exact mechanism of selenium toxicity remains unclear but there are many data about its prooxidant effect particularly in the form of selenite, while selenomethionine and selenocysteine are less toxic. Inorganic forms of selenium reacts with tissue thiols, such as glutathione to form seleno-trisulphides and those are reacting with other thiols to generate oxygen free radicals, such as superoxide anion. Organic diselenides are converted into selenols in presence of thiols which also results oxygen free radical generation. Another free radical hypothesis of selenium toxicosis is based on the methyl-selenide formation, which also results superoxide radicals and induce oxidative stress. Besides free radical formation selenium can have inhibitory effects on thiol proteins, for instance those which have antioxidant affect.

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Trace elements are essential for maintenance of health, growth, and many biochemical-physiological functions of animals and humans (Scott et al. 1982). Among these essential trace elements selenium was discovered by Berzelius in 1818, but its biological significance was not recognized until it was identified as the toxic agent associated with ‘alkali disease’, now termed selenosis, in the United States in 1856 (Franke 1934). Selenium was known as toxic material up to 1957 when Schwarz and Foltz found as essential element for prevention of liver necrosis in vitamin E deficient rats (Schwarz and Foltz 1957) and later the discovery that selenium is the integral part of selenium-dependent glutathione peroxidase enzymes demonstrated a biochemical role for this essential trace element and provided a tool for monitoring its status in animals and humans (Rotruck et al. 1973).

Selenoamino acids, selenomethionine, selenocysteine and selenocystine, are the primary sources of naturally-occurring selenium in plant-based (Burk 1976) and meat-based (Levander 1986) feed and food ingredients. The selenoamino acids are bound in protein, principally as selenomethionine and selenocysteine and constitute 50 to 80% of the total selenium in plants (Butler and Peterson 1967) and in selenium enriched yeast (Kelly and Power 1995). Animals can not synthesize selenomethionine, the primary selenoamino acid, directly from the selenite or selenate forms of inorganic

selenium (Sunde 1990). However, selenocysteine can be found in the body of animals fed inorganic selenium such as selenite and selenate. The presence of selenocysteine is due to synthesis of glutathione peroxidase and other selenoproteins in which the selenocysteine is incorporated. The synthesis of selenocysteine involves a unique process in which selenide is phosphorylated by selenophosphate synthetase to selenophosphate. The selenophosphate is made available to a unique seryl-tRNA^{SEC} that is recognized by selenocysteine synthetase. The selenocysteine synthetase converts seryl-tRNA^{SEC} to selenocysteyl-tRNA^{SEC} that allows insertion of selenocysteine into a peptide chain. The selenocysteine insertion also requires a specific mRNA, an elongation factor, GTP, and the selenocysteine insertion sequence that all interact at the ribosome to read the UGA selenocysteine codon (Low and Berry 1996). Selenomethionine can not be synthesized de novo by animals, therefore it has to supply with feed ingredients and it is easily converted to selenocysteine via cystothionase (Esaki et al. 1981). Selenocysteine can be substituted for cysteine in many proteins, but it is not incorporated directly into specific selenoproteins (Sunde 1990). Selenocysteine is the pivotal amino acid in the synthesis of selenium-dependent cytosolic glutathione peroxidase (Rotruck et al. 1973), but only about 30% of the body’s selenium is incorporated into that enzyme. About 70% of total selenium is incorporated into the other – recently known about 30-50 – selenoproteins in animals and humans (Behne and Kyriakopoulos 2001; Kryukov et al. 2003).

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Table 1. Ability of selenium compounds to generate superoxide in vitro (adapted from Surai 2006).

Superoxide produced	Superoxide not produced
Selenite	Selenomethionine
Selenium dioxide	Selenate
Selenocystine	Elemental selenium
Diselenodipropionate	Selenobetaine
Diphenylselenide	Potassium-selenocyanate

Selenium toxicosis in living organisms

Selenium is toxic for prokaryotes, such as bacteria or algae, however, long-term continuous selenium exposition results selection of selenium resistant strains (Burton et al. 1987). Selenium resistance depends on the capacity of reduction from selenate or selenite to selenide (Oremland et al. 1994).

Among plant species there are accumulators (e.g. *As-tragalus*, *Conopsis*, *Xylorrhiza*, *Oonopsis*, *Stanleya* spp.) which are highly tolerant of selenium (Schrauzer 2003), and non-accumulators which are poisoned by selenium (Smith and Watkinson 1984). The toxicity of selenate and selenite to non-accumulator plants can be attributed to combination of different factors such as the chemical form of selenium (selenate or selenite) and conversion of selenium anions into organic forms or organic metabolites (selenocysteine and selenomethionine). These selenoamino acids act as analogues of essential sulphur compounds, but the physical and chemical differences between selenium and sulphur will result in small, but significant, changes in the biological properties of a selenium-substituted protein (Brown and Shrift 2008). Selenium-tolerant accumulator plants differ from sensitive species, because those contain large quantities of non-protein amino acids, such as seleno-methyl-selenocysteine and seleno-cystathionine or γ -glutamyl-seleno-methyl-cysteine, which are bound selenium and decrease its phytotoxic effect (Pyrzynska 2002). However, these selenoamino acids are rarely detected in non-accumulator plants. In addition, selenium is kept from entering proteins so that the selenium levels in proteins of accumulator plants is significantly lower than the levels in selenium-sensitive plants (Brown and Shrift 2008). Plants also volatilized selenium to dimethylselenide, and volatilization increased linearly with external selenium concentration (De Souza et al. 1998).

Selenium can be toxic for all animals, such as invertebrates (US EPA 1987), fishes (Balogh et al. 2002), amphibians and reptiles (Birge 1978), birds (Green and Albers 1997), mammals (Goehring 1984; McDowell 1997; ATSDR 2003) and humans (Yang et al. 1983; Moeasgaard and Morrill 2002; ATSDR 2003) depending on the dose and duration of intake, and also on its chemical form. Tolerance for selenium toxicity depends on, among other factors, the rate of excretion, and selenium excretion depends on the rate of methylation of selenium as was found in fishes (Hilton et al. 1982).

In vitro studies showed that toxic doses of selenium pro-

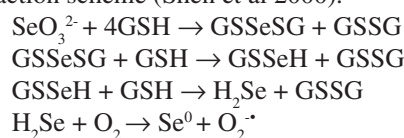
voke cancer (Schrauzer and Ishmael 1974) but there are also selenium-resistant cell lines which showed altered selenium uptake and intracellular glutathione concentrations. The possible mode of resistance is based on two 72 kDa selenium-labelling proteins which are important for the altered selenium uptake (Wu et al. 1995).

In contrary selenium compounds that form the methyl-selenide anion (selenol) have been shown to induce cellular apoptosis even in tumour cells, and one selenium compound, selenium-methylseleno-cysteine, induced apoptosis in cancer cells through activation of capsases, a likely mechanism for other selenium compounds that also induce apoptosis (Ganther 1999; Spallholz 2001).

Prooxidant mechanism of selenium toxicosis

The molecular mechanism of selenium toxicity remains unclear but there is an increasing database that shows the pro-oxidant effect of excess selenium, particularly in the form of selenite (Hafeman et al. 1974; Csallany and Menken 1986, Spallholz 1997; Terada et al. 1999; Raisbeck 2000). However, selenomethionine and selenocysteine, particularly their L-isomers are less toxic than sodium-selenite (Spallholz 1994).

Selenium compounds have different abilities to generate superoxide in vitro as shown in Table 1. Inorganic forms of selenium appear to react with tissue thiols, such as glutathione (Garberg et al. 1988) to form seleno-trisulphides and those are reacting with other thiols to generate oxygen free radicals, such as superoxide anion ($O_2^{\cdot-}$) by redox catalysis (Seko et al. 1989). Selenite reacts with glutathione endogenously in cells or extracellularly causes toxicity by the formation of superoxide and elemental selenium (Seko et al. 1989; Spallholz 1994; Seko and Imura 1997) according to the following reaction scheme (Shen et al 2000).



Organic diselenides (e.g. selenocystine and seleno-cystamine) are converted into selenols (RSeH) in presence of thiols which also results oxygen free radical generation during further reductions catalyzes the formation of superoxide under aerobic conditions in the presence of thiol; this reaction could play a role in the toxicity of diselenides and alkylselenols (Chaudiere et al. 1992).

Those selenium compounds such as elemental selenium (Gao et al. 2000), selenates and seleno-ethers (RSeR), that do not readily form a selenide (RSe-) anion, or selenoenzymes, where selenium is sequestered, therefore do not react with thiols, are non toxic or only after reduction to selenite or selenol (Spallholz 1994).

Another free radical hypothesis of selenium toxicosis is also described (Spallholz and Hoffman 2002). It is based on

the methyl-selenide formation, which also results superoxide radicals and at least oxidative stress. Excess selenium in the form of selenocysteine inhibits the methylation of selenium and increases the amount of intermediary metabolite, hydrogen-selenide, which can also be toxic (Ganter 1979).

Besides free radical formation selenium can have inhibitory effects on thiol proteins, for instance those which have antioxidant affect, by modification via (1) formation of S-Se-S (selenotrisulfides) and S-Se (selenylsulfide) bonds, (2) catalysis of S-S (disulfide bonds) with no incorporation of selenium in the protein, and (3) formation of Se-Se diselenides (Ganter 1999). This catalytic reaction of selenium compounds with thiols likely accounts for selenium toxicity to cells *ex vivo* and *in vivo* where the major glutathione producing organ, the liver, is also the major target organ of selenium toxicity. Selenium also inhibits several thiol-containing enzymes, such as methionine-adenosyltransferase, succinate-dehydrogenase, lactate-dehydrogenase and NADP⁺-isocitrate-dehydrogenase (Nebbia et al. 1990).

The prooxidant activity of selenium may also account for cellular apoptosis and may provide a useful pharmaceutical application for selenium compounds as antibacterial, antiviral, antifungal and anticancer agents (Spallholz 1997; Nilsonne et al. 2006). However, various human carcinoma cell lines (e.g. breast, hepatoma, neuroblastoma and colon carcinoma) showed different sensitivity to selenium derivatives, such as methyl-L-selenocysteine or selenomethionine (Jariwalla et al. 2009).

The produced free radicals are involved in uncontrolled chain reactions, which affect biomolecules, primarily phospholipids, causing lipid peroxidation (Halliwell and Gutteridge 2007). Selenium toxicosis (acute or chronic) turns up when the level of oxidative damage exceeds the capacity of antioxidant defense system, or exceeds the ability of the organism to build the potentially reactive selenocompounds in selenoproteins, or convert them to non-reactive forms (Yan and Spallholz 1993). Selenium toxicosis causes DNA damage (Kelly et al. 1998) by generating 8-hydroxyguanosine DNA adducts (Kim et al. 2004) and lipid peroxidation, and as an effect of the oxidative stress, membranes (e.g. cell-organelle membranes) lose their integrity thus lysosomal enzymes can leak out of them causing serious necrotic damage in tissues (Mézès and Matkovichs 1986; Mézès and Sályi 1994; Balogh et al. 2007). Toxic dose of selenium in form of selenite (6 mg kg⁻¹ body weight per day for 12 days) causes increase the formation of malondialdehyde, as marker of lipid peroxidation, also decreases the amount of reduced glutathione and activities of antioxidant enzymes catalase and superoxide dismutase, but up-regulated glutathione peroxidase in liver of mice (Zhang et al. 2005).

Toxicokinetic studies with chicken embryo showed that toxic doses of selenium reduced the level of lipid peroxides significantly both in hepatic and brain tissues at early hours

after exposure and it was gradually increased thereafter to normal levels later. Further, the effect of selenium on some of the antioxidant enzymes like glutathione peroxidase, glutathione transferase and superoxide dismutase were increased at 6 h post treatment but glutathione levels were reduced in both hepatic and brain tissues (Padmaja et al. 1997). In three weeks-old chicken early period of exposure of excess amount of selenite or selenomethionine increased the level of lipid peroxides and also glutathione peroxidase activity (Balogh et al. 2007).

Detoxification of selenium

Methylation of selenium by both plants (Terry et al. 2000) and animals (Hasegawa et al. 1996) serves to detoxify selenium by generating methylselenides, however excess amount of selenium in the form of selenocysteine decreases the methylation of selenium (Spallholz and Hoffman 2002). Alternatively, full reduction of Se to elemental selenium (Se⁰) as done by some bacteria and the formation of heavy metal selenides such as Ag₂Se or Hg₂Se, results in a non-catalytic non-toxic form of selenium. This catalytic prooxidant attribute of some selenium compounds appears to account for its toxicity when such activity exceeds plant and animal methylation reactions and antioxidant defenses. The excess selenium alternatively can be catabolized into hydrogen selenide and secreted in breath or into trimethyl-selenonium ion and secreted through urine (Ip 1998).

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