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Selenium: Chemical Biology, Toxicology and Radioprotection

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Selenium, a micronutrient and an active constituent of important redox enzymes like glutathione peroxidase (GPx) and thioredoxin reductase (TrxR), has been investigated extensively by researchers all over the world for the last four to five decades. Both inorganic and organic selenium compounds are being evaluated as probable drugs or adjuvants for many viral infections and chronic diseases like cancer. Several clinical trials in cancer patients have confirmed that selenium supplementation helps in recovering from cancer therapy associated side effects and selenium itself does not interfere in cancer therapy. Efforts are on to develop new selenium compounds with anti-cancer properties. These aspects will be discussed in this article.

INTRODUCTION

Selenium, an element discovered in 1817 by John Berzelius, belonging to the group of chalcogens, was initially considered to be a highly toxic element for humans and animals. In the early nineteenth century, selenium was identified as the cause of livestock death in some parts of US due to its high levels in cereal grains. However, subsequent research in early nineteenth century indicated that at small doses selenium may be useful. Finally, Schwarz and Foltz (Schwarz and Foltz, 1957), identified selenium as an essential micronutrient for humans (Fairweather-Tait *et al*., 2011; Lee and Jeong 2012; Weekley and Harris, 2013). Several reports confirmed that selenium deficiency is linked to increased infection risks and is responsible for diseases like Keshan disease – an endemic cardio-myopathy, and Kashin-Beck disease – an endemic osteopathy, primarily observed in China (Chen *et al.,* 1980; Yao *et al.,* 2011). Consequently, the deficiency has also been correlated with the onset of many other disorders such as neurodegeneration, adverse mood states, altered immune response, cardiovascular diseases and cancer

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(Fairweather-Tait *et al*., 2011; Weekley and Harris, 2013). Selenium, is now recognised as an important micronutrient, and is an active centre of important selenoproteins like glutathione peroxidise (GPx) and thioredoxin reductase (TrxR) which play a crucial role in maintaining cellular redox homeostasis (Guo *et al.,* 2007; Trachootam *et al.,* 2008). Selenium compounds are being explored as medicinal for treatment of cancer and other diseases, and also for minimising the side effects of cancer treatment. The current article provides a brief outline of important aspects on chemical-biology, toxicology and radio protective nature of selenium.

Physico-chemical Properties of Selenium in Comparison with Sulphur and Tellurium

Selenium is a member of chalcogens and is present in Group 16 of the periodic table, between sulphur and tellurium, with outer electronic configuration of [Ar] $3d^{10}4s^{2}4p^{4}$. The atomic number is 34 and atomic weight is 78.96. As a chalcogen, it shares many physical and chemical properties with sulphur, however being heavier than sulphur, selenium shows distinct differences. The relative abundance of selenium in earth crust is one-fourth of sulphur, while in humans it is ~10^{5} times lesser. Selenium has lower electro-negativity and ionisation energy and larger atomic radius than sulphur. The bond dissociation energies of C-Se, Se-H and Se-Se are less than that of C-S, S-H and S-S bonds respectively. The selenol (RSeH) is more acidic than thiol (RSH). For example in selenocysteine, pKa of Se-H is 5.43, while that of S-H in cystine is 8.22. Thus at neutral pH, significant difference in the redox behaviour and chemical reactivity of selenium is observed compared to sulfur (Jamier *et al*., 2010; Wessjohann *et al*., 2007). Tellurium, a heavier member of the same group is a rare element on earth with electronic configuration of [Kr] $4d^{10}$ $5s^{2}$ $5p^{4}$, atomic number is 52 and atomic weight is 127.60. Like selenium, tellurium can form analogous organotellurium compounds. However due to its larger atomic size, the Te-C bond is weaker than Se-C bond and therefor no n tellurium containing amino acids is found in nature (Cunha *et al.,* 2009).

The redox behaviour of selenium depends on molecular form and

localization with the neighboring groups (Mugesh *et al*., 2001; Mukherjee, 2010). In living beings selenium is present mainly in the form of selenocysteine and selenomethionine (Whanger, 2002). Selenocysteine, considered as the 21st aminoacid, is more reactive than cystine and is incorporated in proteins by the specific UGA codons (Arnér, 2010; Behne and Kyriakopoulos, 2001; Rahmanto and Davies, 2012). Selenium has variable oxidation states between +6 and –2. In selenocysteine the element is fully reduced with oxidation state of –2, while that in diselenide selenocysteine the oxidation state is assigned to –1. Selenium participates in electron transfer reactions in a similar way as sulfur, however due to higher electropositivity selenium can be easily oxidized as compared to analogous sulfur compound (Iwaoka and Arai, 2013; Jacob *et al*., 2003). In organoselenium compounds selenium can interact with the suitable electron donor/acceptor, which results in non-bonding interaction between selenium atom and heteroatoms like nitrogen and oxygen (Bhabhak and Mugesh, 2010). Such non-bonding interactions in low molecular weight organoselenium compounds like ebselen modulates the GPx like antioxidant activity and toxicity (Mugesh *et al*., 2001). Similarly, selenomethionine is a better antioxidant than methionine, because of stabilization of intermediates during oxidation through hemi-bonding interactions between selenium centered radical and nitrogen atom (Priyadarsini *et al*., 2013).

Assimilation, Deficiency and Toxicity of Selenium

The entry point of selenium in animals is via plants, which absorbs the element in its inorganic form (sodium selenite and selenate) from the soil. In plants, selenium gets converted to organic forms such as methylated low molecular weight selenium compounds and the amino acids such as selenomethionine, selenocysteine, methylselenocysteine and γ-glutamyl-Se-methylcysteine (Whanger, 2002). Animals including humans obtain selenium primarily in the form of selenomethionine by consuming plant products. This selenomethionine acts as a precursor for the synthesis of selenocysteine (Battin and Brumaghim, 2009; Behne and Kyriakopoulos, 2001). Both selenomethionine and seleno-cysteine are analogs of naturally

occurring amino acids, methionine and cysteine respectively. The foods rich in selenium are garlic, cabbage, Brazil nuts, broccoli, etc. Selenite and selenomthionine are added to nutritional supplements to increase selenium levels in the body (Fairweather-Tait *et al*., 2011; Whanger, 2002). Normal serum selenium level differs with age and ranges from 70–187 µg/dL. FDA has ranked selenium as 30th mandatory unit for infant nutrition. Recommended dietary allowance for selenium is 55 µg per day for all healthy adults of both sexes. In domestic farm animals of both sexes, the nutritional functionality of selenium ranges from 40–4000 µg/kg (Dhillon and Dhillon, 1997; Spallholz, 2001). In humans, up to 150 µg per day of selenium is added to overcome selenium deficiency. As per US National Research Council, it is safe to consume 50 to 200 µg per day, although the upper limit is considered to be more conservative. Individual dietary selenium intakes across the world vary significantly. The highest levels of intake have been reported in seleniferous regions of China and Venezuela. Additionally, in special conditions like treatment of viral infected individuals, selenium as high as 2000 µg per day, is recommended (Hou *et al*., 1993; Fairweather-Tait *et al*., 2011). Investi-gations in China indicated that 750 µg per day is safe and did not produce toxicity (Fairweather-Tait *et al*., 2011).

Selenium prevents production of reactive oxygen species (ROS) and also scavenges some of these, thereby decreasing the risk of diseases associated with oxidative stress (Papp *et al*., 2007; Priyadarsini *et al*., 2013). Selenium is necessary for optimal immune response (Zwolak and Zaporowska, 2011). Selenium deficient individuals show decreased IgM and IgG titres. Selenium plays an important role in affecting prostacyclin and thromboxane ratio. Selenium supplementation is considered as a therapeutic remedy to decrease blood clotting.

Higher selenium also leads to severe toxicity, and self-supplementation with frequent and higher selenium may retain large quantities of selenium in the body and therefore all necessary precautions must be undertaken while supplementing with selenium. Increased body selenium leads to selenosis, a condition of acute selenium poisoning in humans, characterized by loss of hair, nails and

swelling at the fingertips. Although Nelson *et al*. (1943) classified selenium as a carcinogen, there is no evidence that selenium is carcinogen in humans. Since then a large number of reports on *in vivo* toxicology of selenium compounds has been reported (Fairweather-Tait *et al*., 2011; Nogueira and Rocha, 2011). However, all the studies emphasized that selenium becomes toxic only if its intake crosses the supplementation limit (> 200 µg/day). The toxicity of selenium compounds not only depends on the quantity of element consumed, but also on its chemical form (Fairweather-Tait *et al*., 2011; Nagy *et al.,* 2015; Painter 1941; Spallholz, 1994). Most of the current knowledge on selenium toxicity has been established by studying the effects of supplementation with inorganic selenite at supra nutritional doses in animal models. The molecular mechanism underlying selenium toxicity is not completely understood. In a few reports, the toxicology of inorganic selenium has been related to the oxidation of thiols of biological importance, producing superoxide and hydrogen peroxide (Fairweather-Tait *et al*., 2011; Kitahara *et al*., 1993; Nogueira *et al*., 2004; Wrobel *et al*., 2016). Organoselenium compounds in general are less toxic compared to inorganic selenium compounds because of the oxidation state and its slow metabolism. However, organoselenium compounds undergoing reductive metabolism induces oxidative stress in cells through consuming glutathione (GSH). Further, the metabolic intermediates of organo-selenium compounds depending on the chemical reactivities can oxidize the vicinal thiol/sulfahydril groups of proteins involved in signal transduction pathways leading to inactivation and subsequent toxicities (McKenzie *et al*., 2002; Nogueira and Rocha, 2011). The classical example of involvement of such mechanisms in organoselenium induced toxicity has been documented in case of diphenydiselenide. In this study, the subcutaneous administration of diphenydiselenide (250 µmol/kg body weight) induced anemia through oxidizing the enzyme delta-aminolevulinic acid dehydratase (δ-ALA-D) involved in hemoglobin metabolism (Jacques-Silva *et al*., 2001). Apart from the above mechanisms, compounds like seleno-methionine exhibit toxicity due to non-specific incorporation in proteins leading to loss

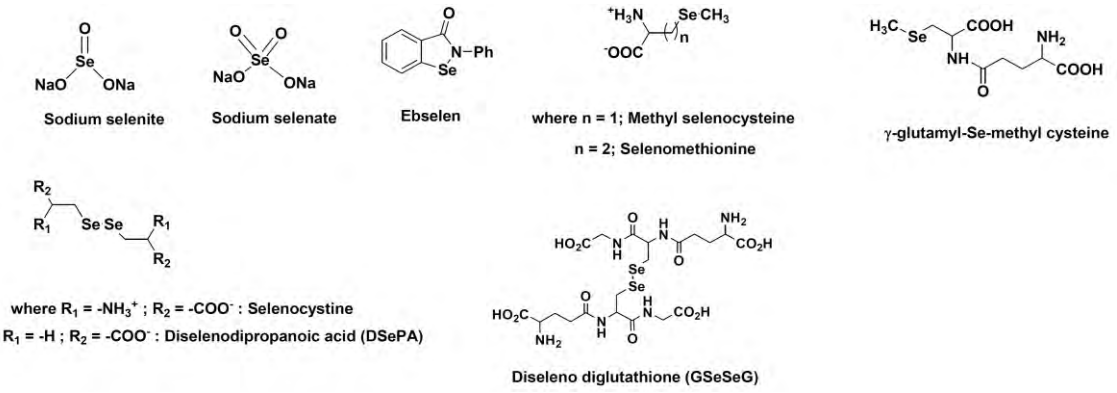

Fig. 1: Structure of important selenium compounds.

of protein functions. Further, acute selenium poisoning leads to cardio-circulatory failure and pulmonary edema (Fairweather-Tait *et al*., 2011). High selenium levels increase the risk of type 2 diabetes and amyotrophic lateral sclerosis (Brozmanová *et al*., 2010; Fairweather-Tait *et al*., 2011)

Selenium Metabolism

Both inorganic and organic selenium compounds are metabolised to hydrogen selenide (H_2Se). Inorganic selenite is reduced to selenide through activation of TrxR and thioredoxin. Alternately selenite (SeO_3^{-2}) reacts with GSH to form selenodiglutathione, which is a substrate/intermediate for reduction of selenoglutathione by glutathione reductase to selenol that reacts with GSH to yield H_2Se (Fairweather-Tait *et al*., 2011; Weekley and Harris, 2013). Selenomethionine is incorporated in proteins in place of methionine non-specifically. Alternately, seleno-methionine and other organoselenium compounds are converted to seleno-cysteine via the intermediacy of seleno-cystathionine. Selenocysteine β-lyase releases H_2Se from selenocysteine. Some compounds are converted to H_2Se through methylselenol (CH_3SeH). H_2Se is incorporated in to selenoprotein P in the liver, which is a source of all selenoprotein synthesis. H_2Se is excreted in the urine in the form selenosugars. Some times H_2Se may be converted to CH_3SeH and $(CH_3)_2Se$ through methyl transfarases and $(CH_3)_2Se$ is excreted in the breath. Selenium metabolites and compounds have been shown to be effective in the detoxification of heavy metals such as Cd, Hg, Pb, As (Tapiero *et al*., 2003). One of the mechanisms suggests formation of biologically inactive selenides which accumulate as

granules in some organs (García-Sevillano *et al.,* 2015; Dauplais *et al.,* 2013). Chemical structures of important selenium compounds are given in Fig. 1.

Selenoproteins

The groups of proteins that contain selenium as an integral part of the polypeptide chain are defined as selenoproteins and these proteins are responsible for most of the physiological functions mediated by selenium such as role in cellular antioxidative protection, redox regulation, male fertility, thyroid function and immune function. Selenoproteins are present in all lineages of life (i.e., bacteria, archaea and eukarya) (Arner, 2010; Weekley and Harris, 2013; Papp *et al*., 2007). In prokaryotes, formate dehydrogenase, hydrogenases and glycine reductase are important selenoproteins in which selenocysteine is present as the selenium moiety. In eukaryotes at least 25 selenoproteins have been identified, and important antioxidant selenoproteins, GPx, TrxR and Selenoprotein P (SelP) are discussed in the following sections.

Gpx

The most important and well-studied selenoprotein in eukaryotes is GPx. It is an antioxidant enzyme that detoxifies peroxides and protects against oxidative stress, and was essential for life in a knockout mouse model. GPx protects biomembranes and other cellular components from oxidative damage by catalyzing reduction of a variety of hydroperoxides, using glutathione (GSH) as the reducing substrate. Four distinct isoforms of GPx have been reported to be expressed in mammals, the classical cytosolic GPx (cGPx), phospholipid hydroperoxide GPx (PHGPx), plasma GPx (pGPx) and gastrointestinal GPx (GI-GPx) (Brigelius-Flohé and Maiorino, 2013). cGPx catalyses reduction of hydrogen peroxide and a limited number of organic hydroperoxides such as cumene hydroperoxide and *tert*-butyl hydro-peroxide. The PHGPx is active on all phospholipid hydroperoxides, fatty acid hydroperoxides, cumene hydro-peroxide, *tert*-butyl hydroperoxide, cholesterol hydroperoxides and hydrogen peroxide (Ursini *et al.,* 1985). Although pGPx and GI-GPx reduce hydrogen peroxide and organic hydroperoxides, these are less active than the cGPx. The four GPx isoforms require selenium in form of selenocysteine in the active sites of GPx enzyme and are directly involved in catalytic reactions.

TRxR

Human TRxR is an important selenoprotein, known for its role in DNA synthesis and protecting cells from oxidative stress (Arner, 2010). It is the only enzyme that catalyses the reduction of oxidized thioredoxin (TRx) using NADPH as a source of reducing equivalents. Trx is a ubiquitously present redox-active peptide, whose major function is to supply reducing equivalents to enzymes such as ribonucleotide reductase involved in nucleotide synthesis (Nordberg and Arner, 2001). During the process, TRx is oxidized and therefore needs to be reduced by TRxR. In addition to TRx, several other endogenous compounds such as lipoic acid, lipid hydroperoxides, cytotoxic peptide NK-lysin, vitamin K, dehydroascorbic acid, ascorbyl free radical and tumour-suppressor protein p53 have been reported as the substrate for TRxR. In mammals, two isoforms of TRxR namely TRxR1 and TRxR2 have been reported. The TRxR1 is localized in the cytoplasm, whereas TRxR2 is localized in the mitochondria. Both TRxR1 and TRxR2 possess selenocysteine at the C-terminal end, which is required for the catalytic activities. The knockout mice model of TRxR1 and TRxR2 suggest that deletion of these enzymes cannot be compensated by any other selenoproteins (Horstkotte *et al*., 2011; Jakupoglu *et al*., 2005).

SelP

SelP is a plasma protein, which contains ten selenocysteine residues per polypeptide chain (Burk and Hill, 2005) accounting more than 50% of the selenium content present in mammalian plasma. The physiological roles of SelP is not completely understood, however its function as an extracellular antioxidant seems most probable. The protective role of SelP in human plasma against the peroxynitrite and its phospholipid hydroperoxide reducing activity are well documented in literature (Rock and Moss, 2010). Some studies suggest a probable role of SelP in intracellular transport and storage of selenium (Renko *et al*., 2008). Although, SelP has been shown to be expressed in various tissues, the plasma level of SelP is mainly contributed by liver (Renko *et al*., 2008).

Apart from selenoproteins some low-molecular weight selenium compounds, such as methylselenic acid, methyl-selenol, methylselenocysteine, and selenomethionine synthesized in the

body as byproduct of selenium metabolism also contribute to physiological functions through the antioxidant mechanisms (Fairweather-Tait *et al*., 2011)..

Selenium and Viral Diseases

Selenium deficiency has been linked with the progression of several viral diseases like Human immunodeficiency virus (HIV) and Coxsackie virus (Fairweather-Tait *et al*., 2011; Rayman, 2012). Recent outbreak of Ebola virus in West Africa has also been linked with selenium deficiency (Abd-ElMoemen *et al.,* 2015). Epidemio-logical studies linked the outbreak of life threatening viral diseases like AIDS and hemorrhagic fever caused by HIV and Ebola viruses with selenium deficiency. However the idea that retroviruses may incorporate selenium of the host cells into viral proteins or "viral selenoprotein theory" has emerged in the last decade. Till date there is no absolute direct proof that a virus can make a selenoprotein. However, computational analysis have not only confirmed presence of several of UGA codons along with the selenocysteine insertion sequence in the genome of HIV and Ebola virus, but also established similarity of some of viral proteins with mammalian GPx (Ramanathan and Taylor, 1997; Taylor *et al*., 1997). The high versus low selenium content within cells acts as a signal for HIV viruses to differentiate between a healthy and a dying cell (Taylor *et al*., 1997). During selenium deficiency, the ROS generation within the host cells is increased leading to oxidative stress, signaling HIV viruses to replicate and leave the dying cell to infect another healthy cell (Taylor *et al*., 1997; 2016). The supplementation of selenium in AIDS patients induces expression of host as well as viral selenoprotein. These seleno-proteins protect the host cells from oxidative stress leading to inhibition of viral replication and spread of infection (Taylor *et al*., 1997; 2016). Since the viral replication is inhibited, the chance of viral genome to undergo mutation and to acquire characteristics of drug resistance is also reduced. In contrast to HIV, Ebola viruses have been postulated to contain genes with several selenocysteine insertion sites (Ramanathan and Taylor, 1997; Taylor *et al*., 2016). Therefore, the growth of Ebola virus in the host cells is expected to compete with the host for

incorporation of selenium in selenoprotein. This may cause a condition called "induced selenium deficiency" in the host cell and can contribute to the blood clotting characteristics of hemorrhagic fever. The above assumption is justified considering that the biochemical basis for an anti-clotting effect of selenium is well established. Clinical data supporting the use of selenium for treatment of Ebola-like hemorrhagic fever, demonstrated the remarkable results. In the study, administration of a very high oral dose of 2 mg selenium per day as sodium selenite, for 9 days reduced the death rate from 100% (untreated) to 37% (treated) in very severe cases, and from 22% to zero in the less severe cases (Hou *et al*., 1993).

Ebselen, an Important Synthetic Selenium Antioxidant

Ebselen, 2-phenyl-1,2-benzisoselenazol-3(2H)-one (PZ 51, DR3305), is an organoselenium compound (Fig. 1), extensively studied and considered as a standard synthetic selenium antioxidant (Azad and Tomar, 2014; Muller *et al*., 1984). It exhibits promising GPx like activity by scavenging hydrogen peroxide and hydroperoxides and also reacts with peroxynitrite. It inhibits enzymes such as lipoxygenases, NO synthases, NADPH oxidase, protein kinase C and H^+/K^+-ATPase. Ebselen exhibits anti-inflammatory and anti-oxidant properties and protects against oxidative challenge, which has been demonstrated in a variety of *in vivo* models (Schewe, 1995). It is a modest immunostimulant and induces several interleukins including IL-1, IL-6, IL-10 and IL-18. Ebselen is less toxic than many other compounds, as metabolism produces compounds in which selenium is retained within the ring structure. Ebselen demonstrates blood-brain permeability and rapid absorption following oral administration (Singh *et al*., 2013). A variety of studies demonstrated that ebselen attenuates neuronal cell death induced by ischemia/reperfusion. It has been shown to be a safer alternative for lithium, for treatment of bipolar disorder. It shows antidiabetic properties and prevents associated heart complications in diabetic rats (Saad *et al*., 2006).

Experimental studies in rats and dogs revealed that ebselen inhibits both vasospasm and tissue damage in stroke

models, which correlates with the inhibitory effects on oxidative processes. Results from randomised, placebo controlled, double blind clinical studies on the neurological consequences of acute ischaemic stroke patients treated with ebselen showed encouraging results. Safety and tolerability were good and no adverse effects were observed. High concentrations of ebselen caused cellular toxicity (Singh *et al.,* 2013).

Selenium in Radiotherapy

Radiotherapy is one of the common treatment modalities for cancer. The approach of radiation therapy is to minimize radiation damage to normal tissue, while maximising radiation exposure to tumor tissue (Weiss *et al.,* 1992). However in practice, normal tissue damage occurs during radiation therapy and therefore it is necessary to protect normal tissue with the help of external agents. Radioprotectors are therefore developed and even after several years of research, the only clinically acceptable radioprotector till date is amifostine, an aminothiol, with the active group of RSH (Andreassen *et al*., 2008). Since selenium belongs to the same group as sulphur in the periodic table, selenium compounds both in inorganic and organic forms have been tested for radioprotection. It has been reported that radiotherapy significantly reduces selenium levels (Eroglu *et al*., 2012). Sodium selenite was the first selenium compound tested for radioprotection in mice. When administered intraperitoneally before (–24 h and –1 h) or shortly after (+15 min) irradiation, it increased the thirty-day survival of mice irradiated at a lethal dose of 9 Gy (Weiss *et al*., 1992). Selenite was also effective when administered in combination with vitamin E before γ-irradiation and prevented radiation induced reduction in levels of antioxidant enzymes in mice model. In these models, sodium selenite supplementation increased the activity of serum GPx and reduced the therapy induced oxidative stress. Selenite has also been reported to exhibit radioprotection in normal fibroblast cells, but not in head and neck carcinoma cells. Effect of selenite supplementation in cancer patients undergoing radiotherapy for gastrointestinal, breast, lung, larynx, head and neck, non-Hodgkin lymphoma, brain, prostrate and gynaecological cancers was studied (Lippman *et al*.,

2009; Klein et al., 2011). Sodium selenite was administered orally in the dose range of 200–500 μg daily or 1000 μg daily by infusion. Based on the results from 16 clinical trials, it was concluded that selenium supplementation improved the general condition of the patients and at the doses employed, selenium toxicity was not observed, and did not decrease effectiveness of radiotherapy (Puspitasari *et al*., 2014).

A few organoselenium compounds like selenourea, selenocysteine, seleno-xanthene, and selenomethionine, have also been examined for radioprotection using *in vitro* and *in vivo* models. However these agents did not show promising activity, except seleno-methionine, which significantly increased the 30-day survival of mice irradiated at lethal doses of 9 and 10 Gy (Weiss *et al*., 1992). It was equally protective when administered at 24 h, 1 h and 15 min prior to γ-irradiation. However, when selenomethionine was provided in the diet as selenous yeast protection against acute or chronic radiation exposure was not observed. Both selenite and selenomethionine showed remarkable chemo-preventive activities in human clinical trials, the former exhibiting better activity. Ebselen has also been tested for radioprotection in mice. The results indicated that ebselen administration for 14 days at a daily dosage of 10 mg/kg body weight before whole body irradiation at 8 Gy provided substantial protection (60%) against mortality and oxidative damage (Tak and Park, 2009). Thus results reported from various laboratories support that selenium compounds have a potential as radioprotectors. Since selenium in organic form exhibits lower toxicity, than in inorganic form, extensive research on modulation of radiation induced changes by new organoselenium compounds is required.

Our group has also contributed in this research area. After evaluating a number of in-house synthesized organoselenium compounds like selenoethers, diselenides and cyclic selnolanes, 3′-3′ diseleno-dipropionic acid (DSePA (Fig. 1) was identified as a lead radioprotector. It is a water soluble derivative of seleno-cysteine, synthesized in our laboratory. It was shown as an effective free radical scavenger, anti-hemolytic and GPx mimic (Kunwar *et al*., 2007). The compound was also nontoxic in normal cells and maximum tolerable dose

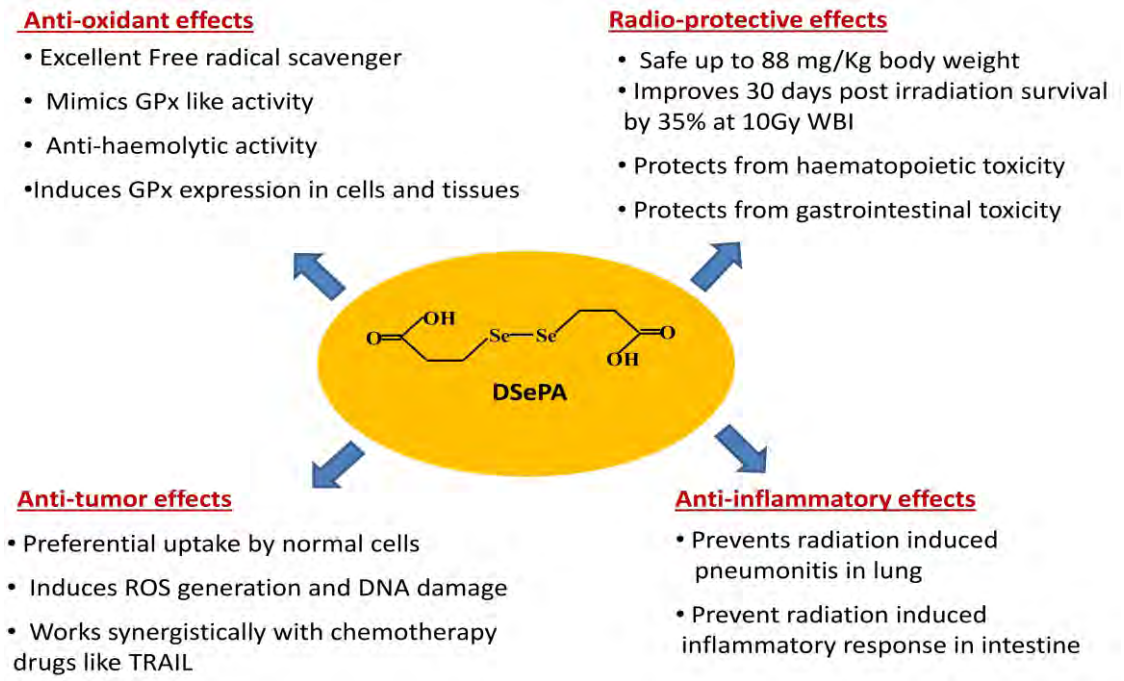

Figure 2: Potential biological activities of DSePA.

(MTD) in mice was estimated as ~88 mg/kg body weight for intraperitoneal mode of administration (Kunwar *et al*., 2010). The radio-protective ability of DSePA was evaluated in BALB/c mice, wherein the administration of DSePA (2 mg/kg/day for 5 consecutive days) prior to whole body irradiation (10 Gy) led to 35% increase in the number of mice surviving up to 30 days. Our results demonstrated DSePA treatment to prevent oxidative damage (such as lipid peroxidation and DNA damage), apoptosis and inflammatory response in radiosensitive organs like hematopoietic and gastrointestinal system (Kunwar *et al*., 2010; 2011). Since toxicological evaluation is a prerequisite for any compound to be proposed for clinical application, DSePA was investigated in detail for the same in Chinese Hamster Ovary cells. The results of this study indicated that DSePA treatment on its own did not induce toxicity, but prevented radiation induced genotoxicity and subsequent cytotoxicity in model cellular systems (Chaurasia *et al*., 2014). DSePA also protected against the depletion of endogenous antioxidants in hepatic tissue of irradiated mice. In line with these observations, DSePA improved the 30-day survival of the irradiated mice by 35%.

Late lung tissue responses like

pneumonitis and fibrosis are the serious dose-limiting side effects of thoracic radiotherapy used in several malignancies affecting organs in the thorax area. Encouraged by the preliminary results on DSePA, we showed that administration of DSePA during the post irradiation period at a similar dosage (2 mg/kg/three days in a week) delayed the thoracic radiation (18 Gy) induced pneumonitis response in C3H/HeJ mice (Kunwar *et al*., 2013). The DSePA treated mice had significantly reduced levels of lipid peroxidation and inflammatory cell influx in the lungs and increased GPx, compared to mice receiving only irradiation. Further pharmacokinetic studies of orally administered DSePA in different organ systems of tumor bearing mice showed maximum bioavailability of DSePA in lungs followed by kidney, liver and intestine; while, that in the tumors was significantly low (Gota *et al*., 2015). Hence, the preclinical investigations are extended to develop DSePA as oral supplemented lung radioprotector for thoracic radiotherapy. The studies related to DSePA are summarized in Fig. 2.

Pro-oxidant Effects of Selenium and Use in Cancer Therapy

Similar to several trace elements, selenium also plays a dual role. It is an antioxidant at low concentrations (< 200 µg/per day) and acts as a pro-oxidant at higher concentrations (> 200 µg/per day) (Fairweather-Tait *et al*., 2011; Lee and Jeong, 2012). The antioxidant function is mainly through selenoproteins, which maintain the intracellular redox homeostasis and thereby preserve the normal physiological processes in the cell; while at high concentrations, it becomes a source of ROS. The pro-oxidant behaviour at high concentration suggested selenium as a probable anti-cancer drug. Several researchers studied the anticancer effects of selenium compounds. The pro-oxidant effect of selenium was correlated with well-established growth inhibiting and cytotoxic activities through cellular proteins like AP-1, NF-kB, P53 and protein kinase C under *in vivo* conditions (Brozmanová *et al*., 2010; McKenzie *et al*., 2002). The effects are more pronounced in malignant cells than normal cells, indicating potential candidates for anticancer agents

(Fernandes and Gandin, 2015; Orian and Toppo, 2014).

Both inorganic and organic selenium compounds have been evaluated for anticancer activity. Sodium selenite showed significant cytotoxicity in micromolar concentrations in a variety of cancers cells from different organs such as lung, prostrate, cervix, ovary and colon (Brozmanová *et al*., 2010; Fairweather-Tait *et al*., 2011). It was also effective against drug resistant cells and enhanced efficacy of other cancer drugs like 5-fluorouracil, oxaliplatin and irinotican in *in vivo* models. However there were some reports indicating genotoxicity of selenite (Nogueira and Rocha, 2011). A recent clinical study in 34 cancer patients was reported. The patients received first line chemotherapy, followed by selenite treatment daily for five days over two or four weeks. The results concluded that sodium selenite is safe and tolerable when administered up to 10.2 mg/m^2, no major systemic toxicity was reported and the most common adverse effects were fatigue, nausea, and cramps in fingers. The study indicated that the drug was an effective chemotherapeutic agent which works in three ways, either by itself, or in combination with other chemotherapy drugs, or as a remedy for chemotherapy toxicity (Micke *et al.,* 2009; Misra *et al*., 2015).

Organic selenium compounds have been examined for anticancer activity (Fernandes and Gandin, 2015). These include selenomethionine, methylselenocysteine, selenoglutathione, selenocysteine, aromatic selnides, quinozoline derivatives, diphenyl diselenide, ebselen, etc. However most of the studies were limited to in vitro studies and examined in different cancer cell lines. Several of them showed encouraging results but a lot more studies under *in vivo* conditions are necessary to explore their ability against cancer. Selenocysteine is the only compound which has been extensively studied and evaluated. It is a diselenide of the amino acid, selnocysteine (Chen and Wang, 2008). It has been shown to be effective against human melanoma, cervical and lung cancer cells. It also showed selectivity between cancer and normal cells, in melanoma cells, selenocystiene potentiated the efficacy of 5-fluorouracil (Fan *et al*., 2013). However, it has limitation due to low water solubility and instability.

It is also interesting to note that

DSePA (Kunwar *et al*., 2010) which shows significant radioprotection in normal cells can also be used in chemo-sensitization of melanoma cancer cells (Cao *et al*., 2014). Combinatorial treatment of DSePA with tumor necrosis factor related apoptosis inducing ligand (TRAIL), a promising targeted cancer drug, not only enhanced the apoptotic inducing efficacy of TRAIL but also overcame resistance of melanoma cells. The ability of DSePA to chemo-sensitize melanoma cells was through the induction of ROS, DNA damage and P53 activation. The fact that DSePA shows antioxidant activity in normal cells and pro-oxidant activity in tumor cells, makes it an ideal candidate for future evaluation as a cancer adjuvant drug.

CONCLUSIONS

Selenium, an element considered to be highly toxic, is now considered as an essential nutrient. Selenium deficiency has been linked with several diseases where oxidative stress plays an important role. Selenium exerts its effects through modulation of redox regulating seleno-proteins. Many selenium compounds, both inorganic and organic forms, show excellent antioxidant effects, and several exhibiting promising effects as radio-protectors. Excessive selenium leads to toxicity, a property to be considered for design of new selenium based anticancer drugs. With increasing understanding of the chemistry and biology of selenium, it is feasible that new selenium compounds may be used as therapeutic agents.

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CONFLICT OF INTEREST

The authors claim no conflict of interest.

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