Organoselenium Compounds: A New Generation of Radioprotectors

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Abstract

Exposure of living organisms to ionizing radiation can cause health hazards and radioprotectors are employed to minimize such unwanted effects. Over the years a number of sulfhydryl compounds have been examined for radioprotection and only one agent, amifostine, is approved in the clinic. Selenium, a higher analogue of sulfur, is a micronutrient and a constituent of redox enzymes like glutathione peroxidase (GPx). With an aim to develop less toxic, GPx active, organoselenium compounds, as potential radioprotectors, we initiated a programme on synthesis and radioprotection studies of organoselenium compounds. In this article, the current status of research on selenium compounds as radioprotectors is presented.

Radiation exposure and Radioprotectors

Ionizing radiation, both high-energy electromagnetic and charged particles, has a wide spread usage in medicine, agriculture, energy, food storage, etc. and poses health hazards when used improperly. Exposure of living organisms to radiation can induce a number of abnormalities like mutation, cancer, and even death[1]. When radiation interacts with living cells, water being the major constituent (~70%), undergoes radiolysis producing highly reactive free radical species, inducing oxidative stress[1,2]. Cellular damage initiated by these radical species is the origin of tissue and organ injury [3]. Depending on the type, exposure and linear energy transfer of the radiation, all these events occur within hours to weeks. Sometimes delayed and chronic effects are observable even after many months of exposure.

The extent of radiation injury depends on the absorbed dose, expressed in the units of Gray (Gy), equal to one joule of energy deposition in one kilogram (1 J/kg) of the material. In addition to this, sensitivity of different organs equally determines the manifestation of radiation injury. For example, organs like brain, bone, muscle, thyroid, pituitary, adrenal and liver are radio-resistant, whereas others like lymphoid organs, reproductive organs, bone marrow and intestinal crypts are radiosensitive[1-3]. Cellular oxygen enhances the radiation damage and reduced oxygen levels, as observed in certain hypoxic tumors, make them radioresistant[2].

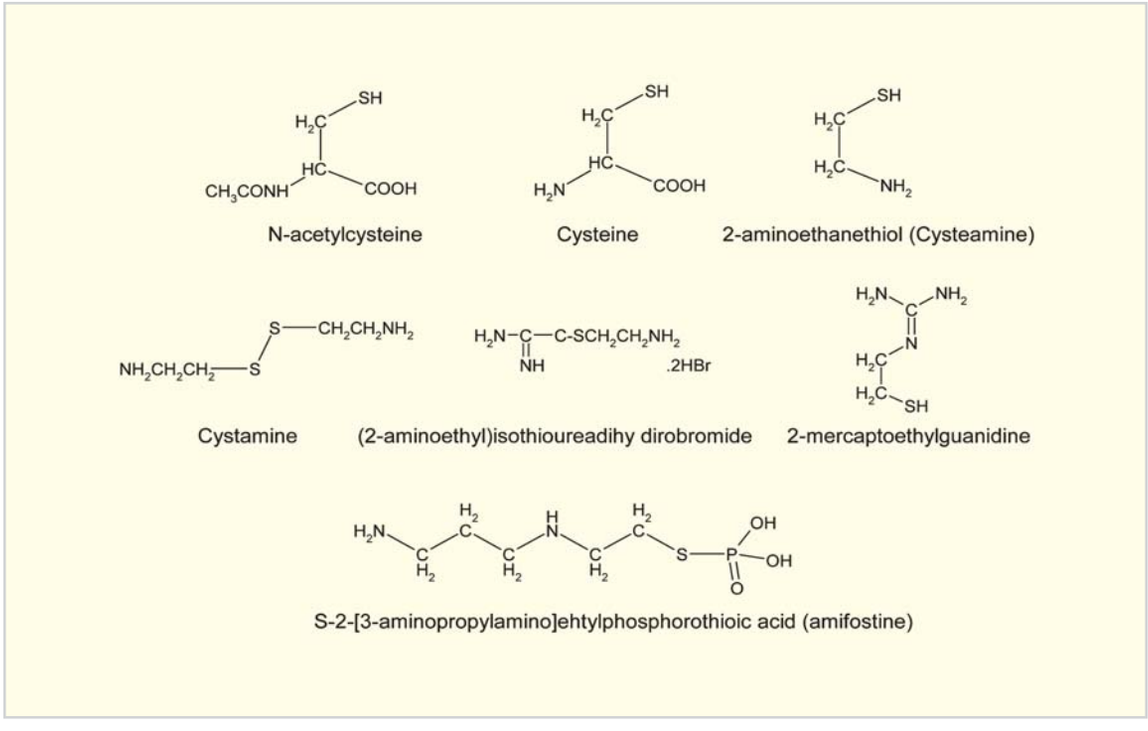
A radioprotector is a chemical substance or a mixture of compounds, capable of minimizing the damaging effects of ionizing radiation to normal tissue[4]. Development of radioprotectors has been an area of active research from the beginning of the nuclear era. With the recognition that normal tissue protection during radiotherapy is as important as the destruction of the cancer cells, the focus of radioprotection research became more therapy oriented. However, an ideal radioprotector should be able to protect against the deleterious effect of any type of radiation during therapeutic procedures as well as during nuclear accidents. Additionally, a radioprotector should be inexpensive, have no toxic implications, and can be orally administered, with

rapid absorption and a reasonably good Dose Modification Factor (DMF). The efficacy of any agent to act as a radioprotector is evaluated in animal models using distinct end-points like survival against radiation-induced lethality, protection to hematopoietic or gastrointestinal (GI) systems, and mutagenesis [3,4]. The most commonly compared parameter, DMF, varies with the type of radiation, dose rate, administered dosage of radioprotector, time and schedule of treatment, animal strain, etc.

Over the years, several sulfhydryl compounds, such as *S*-2-[3-aminopropylamino] ethylphosphorothioic acid (amifostine), cysteine, N-acetylcysteine, cysteamine, cystamine, aminoethylisothiourea dihydrobromide and mercaptoethyl guanidine (scheme 1), have been screened for their radioprotective ability[3,4]. Out of these, the most effective one, and the only agent approved by the FDA for use in protection of normal tissues in patients treated with radiation is amifostine[5]. It exhibits multiple biochemical properties like free radical scavenging activity and high affinity for DNA. Although amifostine is a clinically approved radioprotector, its considerable toxicity at radioprotective doses warranted search for effective and non-toxic alternate drugs[4,6].

Selenium compounds as antioxidants and radioprotectors

Selenium is an essential trace element for both animals and humans and the recommended nutritional dose of selenium for normal humans is 50–60 µg/day[7]. Sodium selenite, selenomethionine, selenium enriched yeast, broccoli, mushrooms, garlic, fish, cabbage, whole grains, wheat, etc. act as selenium supplements. The plants belonging to the genus *astragalus* were found to contain high levels of selenium (several thousand part per million). Selenium deficiency has been implicated in several diseases and some studies have also correlated it with the incidence of cancer. Selenium enters the body through plants, which absorb it from the soil. Inside plants, inorganic selenium is converted to low molecular weight amino acids like selenomethionine (scheme 2). In the body,



Scheme 1: Some selected sulfur compounds used as radioprotectors

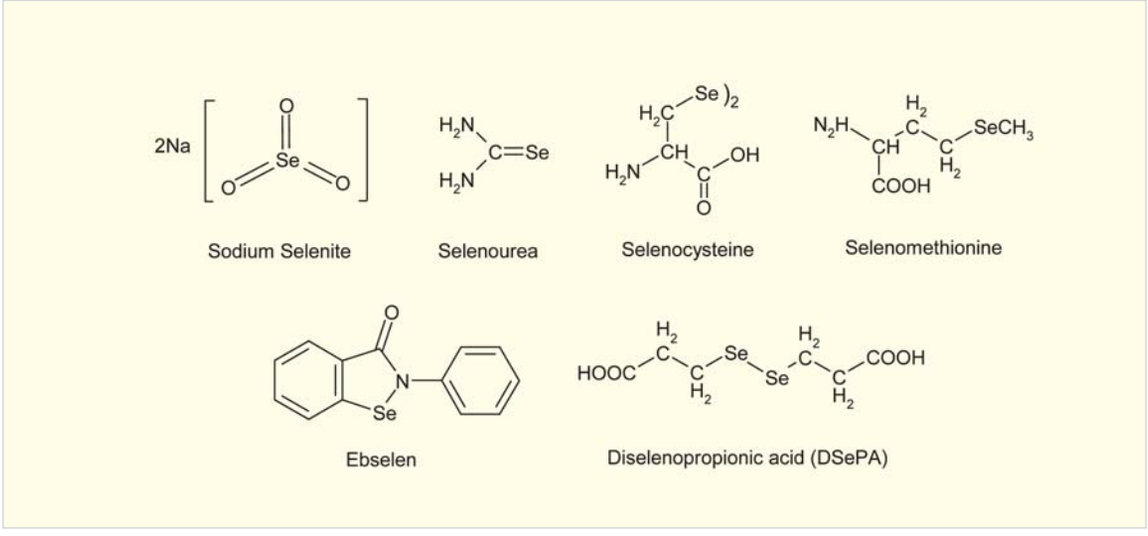
selenomethionine acts as a precursor for the synthesis of selenocysteine, which is the active component of selenoproteins, present in many lineages of life[7]. These proteins are responsible for most of the physiological functions mediated by selenium such as antioxidative action, redox regulation, immune function, etc. In humans, at least 25 selenoproteins have been identified so far. The most important and well-studied selenoproteins is glutathione peroxidase (GPx). It is an antioxidant enzyme that detoxifies peroxides, by converting them to either water or less reactive species.

Compared to its lighter analogue sulfur, selenium is much less abundant in cells[7,8]. Although the general properties such as ionic radii, electronegativity of sulfur and selenium are similar, they show difference in polarizability (2.9 Å^3 for sulfur, 3.8 Å^3 for selenium). The widely varying pKa values of $H_2NCH(COOH)CH_2$-EH (E = S or Se; 8.3 for SH and 5.2 for SeH), make selenocysteine much stronger nucleophile and better reductant than cysteine at physiological pH[8]. These differences enhance the redox reaction rates of selenium compounds with reactive oxygen species. All these advantages of selenium over sulfur prompted researchers to speculate that selenium compounds may be explored as new class of antioxidants and radioprotectors.

Interestingly the thyroid gland, which is radioresistant, has high selenium contents and about eleven selenoproteins are expressed in the gland.

A few selenium compounds in both inorganic and organic forms have been evaluated for radioprotection[4]. Sodium selenite was the first selenium compound tested for radioprotection in mice. When administered intraperitoneally (i.p.) before (-24 h and -1 h) or shortly (+ 15 min) after irradiation, it increased the 30-day survival of mice irradiated at 9 Gy (DMF = 1.1). Further, its injection 24 hours before amifostine treatment decreased the lethal toxicity and enhanced the radioprotective effect of amifostine significantly. Selenite was also effective when administered in combination with vitamin E before γ-irradiation and prevented the radiation-induced reduction in levels of antioxidant enzymes[4]. It showed a significant protective effect during the initial treatment phase of fractionated therapy. Recent scientific research has also indicated that selenite exhibits differential radioprotection. Based on all these studies, selenite was even tested in clinic at a dosage of 500 μg per day, where it reduced the side effects of radio-chemotherapy in head and neck cancer patients[9].

A few organoselenium compounds like selenourea, selenocystine, selenoxanthene, and



Scheme 2: Selenium compounds examined for radioprotection

selenomethionine, have also been examined for radioprotection using *in vitro* and *in vivo* models[4,10]. However, these agents did not show much promising activity, except selenomethionine, which has significantly increased the 30-day survival of mice irradiated at lethal doses of radiation. It was equally protective when administered at 24 h, 1 h and 15 min prior to γ-irradiation[4]. However, when selenomethionine was provided in the diet as selenous yeast it showed no protection against acute or chronic radiation exposure. Both selenite and selenomethionine showed remarkable chemo-preventive activities in human clinical trials, the former exhibiting far better activity. Very recently, a synthetic organoselenium compound ebselen, a well-studied GPx mimic, 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[11]. The results reported from various labs support the argument that selenium compounds have great potential to be developed 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.

Table 1: Investigations related to the radioprotective efficacy of DSePA

Investigations		Systems monitored	Summary of Results	Reference
GPx-like activity `		Enzyme kinetics specificity to hydroperoxide and thiol	Acts as a GPx-mimic with	13
In vitro antioxidant activity		Inhibition of reactive oxygen species induced haemolysis in human RBCs	Inhibits lipid peroxidation of RBC membrane, loss of hemoglobin and K^+ ions .	15
In vitro radioprotection studies		Protection to biomolecules like DNA, lipids and proteins DNA, peroxidation of lipids and oxidation of proteins	Inhibits radiation induced strand break formation in	14
Acute toxicity studies		Swiss albino mice; Mode of injection: intraperitoneal (i.p.)	Maximum tolerated dose = 8.86 mg/kg	17
In vivo radioprotection studies	Hepato (liver) protection	Modulation of antioxidant levels and oxidative stress (5 Gy)	Prevents oxidative stress and depletion of antioxidant enzymes and restored the normal hepatic function and architecture	17
DSePA dosage: 2mg/kg body weight, i.p. five days prior to irradiation	Hematopoietic protection (5 to 7 Gy)	1) DNA damage in leukocytes 2) Spleen parameters and cell death	Inhibits DNA damage in blood leukocytes, and improved spleen index	17
	Gastrointestinal (GI) protection (7 Gy)	Oxidative stress in small intestine and death of epithelial cells	Reduced oxidative stress and prevented morphological changes and apoptosis in intestinal tissue	18
	Survival studies (9 Gy)	30 days survival	Improved survival of mice by 35%	17
	Immuno-modulation (7 Gy)	1) Intestinal inflammation 2) Cytokines in serum	Ameliorated intestinal inflammatory response and restored immune balance.	18

Radioprotection studies on organoselenium compounds from our laboratory

Our group has initiated a project on development of low molecular weight water-soluble organoselenium compounds as radioprotectors. A number of organoselenium compounds new as well as previously reported were synthesized in our laboratory[12]. To enhance the water solubility, functional groups like -OH, -NH_2, -COOH were incorporated in these molecules.

Further, the compounds were examined for free radical reactions, GPx mimicking ability and antioxidant activity. In vitro studies performed on all these compounds indicated that organic diselenides substituted with carboxylic acid functional group are very effective as antioxidants due to less toxicity, ability to scavenge oxidizing free radicals and preventing membrane peroxidation[13-15]. Therefore, diselenodipropionic acid (DSePA), a diselenide, was selected for *in vitro* and *in vivo* radioprotection studies (Table 1). DSePA participates in electron transfer reactions, and the active intermediates produced in the redox reactions can get incorporated in to the GPx enzyme catalytic cycle of DSePA. It scavenges reactive oxygen species very efficiently and in vitro studies confirmed its potential antioxidant and radioprotecting ability[13-16].

The maximum tolerable dose (MTD) of DSePA in mice was found to be 8.82 mg/kg body weight[17]. Therefore, for *in vivo* radioprotection studies, a non-toxic dose of 2mg/kg body weight, of DSePA was injected intraperitonially (i.p.) in mice for five days and one hour after the last dose, the mice were exposed to whole body γ-radiation of ~9 Gy. Their survival was monitored for 30 days daily and compared with that of radiation and DSePA control animals. There was a significant 35% improvement in the survival of animals treated with DSePA and exposed to radiation as compared to radiation control animals[17]. No death was observed in animals treated with DSePA alone. From this study, the dose modification factor (DMF) was estimated to be 1.1. Further DSePA showed significant protection to radiosensitive organs like gastrointestinal (GI) tract, and hematopietic system, as shown in Figure.1[17,18]. In the irradiated mice, DSePA maintained the villi height and villi/circumference of the small intestine and prevented epithelial cells (intestinal lining) from undergoing apoptosis. DSePA also exhibited prophylactic action in the hepatic system of

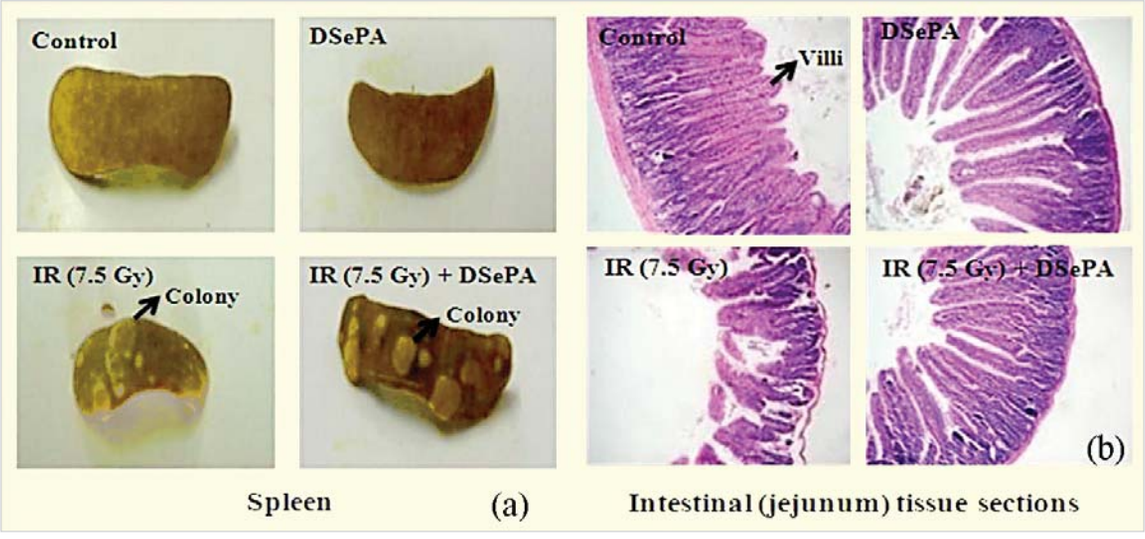


Fig. 1: Pretreatment with DSePA (i.p.) at a dosage of 2 mg/kg body weight protected hematopoietic system and gastrointestinal system as shown by spleen colony formation (A) and maintenance of villi height and numb circumference (b) respectively

irradiated mice through maintenance of the antioxidant enzymes (e.g. GPx) and hepatic architecture. Similarly, DSePA prevented DNA damage in peripheral leukocytes in mice exposed to radiation. Additionally DSePA ameliorated the radiation-induced inflammation and restored the immune balance in irradiated mice.

In conclusion, our *in vitro* and *in vivo* studies on organoselenium compounds revealed that DSePA, a low-molecular weight water-soluble compound with a very low cytotoxicity, is a promising candidate as a radioprotector. Therefore, our future studies are directed to test this compound for tumor selectivity in irradiated animals followed by different phases of preclinical and clinical evaluation. Further, efforts will also be made to design new synthetic derivatives with improved radioprotecting ability.

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