Top Med Chem (2016) 17: 87–118
DOI: 10.1007/7355_2015_86

Published online: 6 June 2015

Selenium-Based Drug Design

Sheldon W. May

Abstract The biochemistry, pharmacology and epidemiology of selenium and selenium-containing compounds continue to be subjects of considerable interest from the viewpoint of public health. Selenium has a long history of association with human health and disease, and we now recognize that this element is an essential nutrient that is critical to key cellular processes. We now know that the selenoproteins constituting the human selenoproteome are encoded by 25 genes in the human genome, and much progress is being made in our understanding of selenium metabolism and the health effects of selenium metabolites in normal and disease states. The idea that selenium-containing dietary supplements might be effective in preventing disease has gone through both optimistic and pessimistic phases in recent years, and the future prospects for such a nutritional approach are unclear at the present time. In contrast, a significant number of promising efforts are underway that are aimed at designing and developing pharmaceutical agents that are selenium-based or that target specific aspects of selenium metabolism. This chapter focuses on some of these efforts to develop new selenium-based anticancer, antioxidant, antihypertensive, antiviral, immunosuppressive, and antimicrobial agents. While most of the efforts that entail designed organoselenium compounds – as opposed to inorganic selenium metabolites – are still at the preclinical stage, evidence is emerging that selenium-based compounds can operate via several beneficial biochemical and pharmacological mechanisms. Since our understanding of the biology, biochemistry and pharmacology of selenium and selenoproteins is rapidly expanding, we can anticipate that the coming years will bring further development of new selenium-based pharmaceutical agents with therapeutic potential against human diseases.

S.W. May (✉)
School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332-0400, USA
e-mail: sheldon.may@chemistry.gatech.edu

Keywords Anticancer agents, Antihypertensives, Antimicrobials, Antioxidants, Antiviral agents, Ebselen, Glutathione, Phenylaminoethyl selenides, Selenazolidines, Selenium, Selenium-based drugs, Selenium-containing metabolites, Selenoproteins, Therapeutic use of organoselenium compounds, Thioredoxin reductase

Contents

1 Introduction

The biochemistry, pharmacology, and epidemiology of selenium and selenium-containing compounds continue to be a subject of considerable interest, particularly from the viewpoint of public health. Selenium has a long history of association with human health and disease [1, 2], and specific populations have historically suffered from certain diseases that are now recognized to be associated with selenium deficiency. A widely known example is Keshan disease, which is characterized by congestive heart failure [3, 4]; healers in Keshan, China, where the disease was discovered, have traditionally treated it with the common herb, *Astragalus*, several species of which accumulate selenium from the soil. Ironically, *Astragalus* may be the very plant seen by Marco Polo in the "Succuir" district of China; the explorer describes how beasts of burden unaccustomed to the area would feast on this plant, thereby causing the animals to become so ill that their hooves dropped off [5]. Polo's description fits what is now known to be an equestrian form of selenosis. Thus, in retrospect, it is likely that even this very early report underscores the critical importance of managing the proper dose and molecular form of selenium when considering therapeutic applications.

It is worth noting that the notion that selenium may exert a protective effect against human cancer was actually discussed in the mainstream scientific literature more than four decades ago [6, 7]. Early geographical studies suggested an inverse correlation between selenium levels and cancer incidence, and cancer mortality rates were found to be significantly lower in US counties with intermediate or high

selenium levels as compared to counties with low selenium levels [8]. Similarly, low selenium concentrations were found in the sera of patients with pancreatic carcinoma and in the plasma of breast cancer patients, and selenium compounds have exhibited antitumorigenic activities in a number of animal studies over the years [5].

Turning to the present time, we now know that selenium (as selenocysteine) is an essential component of the active sites of the enzymes glutathione peroxidase and mammalian thioredoxin reductase and is also present in a variety of other mammalian selenoproteins [9–13]. Both glutathione peroxidase and thioredoxin reductase catalyze reactions that are essential to the protection of cellular components against oxidative and free radical damage. In epidemiological studies, a low concentration of selenium in plasma was identified as a risk factor for several diseases including cancer, cardiovascular disease, osteoarthritis, and AIDS [3, 14–19]. In the United States, the National Academies of Science Institute of Medicine has issued a dietary reference intake report on selenium and other antioxidants [20], with the current recommended dietary allowance (RDA) for selenium being 55 micrograms for adult men and women. It should be noted that symptoms of selenosis appear when dietary levels of selenium exceed 1 milligram/day, whereas a selenium dietary intake below 1 microgram/kg can lead to selenium deficiency diseases such as the aforementioned Keshan disease.

Over the past three decades, there have been a number of efforts aimed at designing and developing pharmaceutical agents that are selenium based or that target specific aspects of selenium metabolism (for some relevant reviews, see [5, 9–12, 21]). These efforts have focused on the design of selenium-based antihypertensive, anticancer, antiviral, immunosuppressive, and antimicrobial agents. There has also been much interest in the development of organoselenium compounds capable of reducing oxidative tissue damage and edema. This chapter will focus on some of the more promising of these efforts to develop new selenium-based therapeutic agents.

It is very important to mention at the outset that concurrent with these drug design and development efforts, some findings that looked initially quite promising emerged from nutritional trials and epidemiological studies. In 1996, Clark and coworkers [22] reported that their long-term, double-blind, placebo-controlled study demonstrated significant reductions in incidences of lung, colorectal, and prostate cancers in patients receiving daily 200 microgram oral doses of "selenium yeast." Subsequently, a prospective study of 33,737 men reported [14] that individuals with the highest selenium levels had only about one-third the likelihood of developing advanced prostate cancer as did individuals with low selenium levels, and similar results were reported in other prospective cancer studies (see, e.g., van den Brandt et al. [15]). In view of findings such as these, the Selenium and Vitamin E Cancer Prevention Trial (SELECT) was launched [23] by the National Cancer Institute. SELECT was designed to be a very large, randomized, placebo-controlled trial of selenium (200 μg/day L-selenomethionine) and/or vitamin E (400 IU/day racemic α-tocopheryl acetate) supplementation for a minimum of 7 years (maximum of 12 years) focusing on the prevention of prostate cancer by these agents.

Unfortunately, SELECT was discontinued since an independent review of the data being collected showed that selenomethionine and vitamin E, taken together or alone, were not preventing prostate cancer [24, 25]. Nevertheless, despite this disappointing result, there still remains much interest from a nutritional perspective in the development of effective selenium supplementation protocols, if only the proper selenium formulation and dosage could be identified (see, e.g., the "post-SELECT" perspective by Ledesma et al. [24]). A detailed discussion of epidemiological and nutritional aspects of selenium supplementation is beyond the scope of this review.

2 Phenylaminoalkyl Selenides

This author's laboratory has developed a family of phenylaminoalkyl selenides [26–31] and we have shown that these selenides possess the ability to propagate a cycle that depletes reduced ascorbate within adrenergic vesicles. Initially, dopamine-β-monooxygenase (DBM) present within the vesicle converts these selenides to the corresponding selenoxides; however, the product selenoxides are then nonenzymatically reduced back to the selenides, with the concomitant and stoichiometric oxidation of reduced ascorbate present in the vesicle to fully oxidized ascorbate. This selenide/ascorbate cycle is a localized process since DBM is present only in these vesicles and reduced ascorbate does not cross the vesicle membrane. While adrenergic vesicles possess a cytochrome b561-dependent ascorbate recycling system, this native system can only recycle semidehydroascorbate, which is generated during DBM turnover, and cannot recycle the fully oxidized ascorbate produced by the nonenzymatic selenoxide/ascorbate reaction. Thus, the net result of selenide processing in the vesicle is the effective local depletion of reduced ascorbate – an essential cofactor for DBM – and the consequent diminution of norepinephrine production. We demonstrated this turnover-dependent ascorbate depletion process both in vitro and in chromaffin granule ghosts, and we confirmed cellular and vesicular uptake of the selenides. Moreover, as predicted on the basis of the redox potentials of selenoxides versus sulfoxides, we showed that while sulfur-containing analogs undergo DBM-catalyzed sulfoxidation, they are not capable of propagating such a cycle of ascorbate depletion. Thus, it is clear that the ability of phenylaminoalkyl selenides to effect turnover-dependent depletion of local reduced ascorbate is a direct consequence of the redox chemistry of the selenium moiety present in these compounds (Fig. 1).

The pharmacological testing of phenylaminoethyl selenide (PAESe) confirmed that this compound exhibits dose-dependent antihypertensive activity when administered i.p. to spontaneously hypertensive rats, and we have provided evidence that the adrenergic nerve terminal is indeed the pharmacological site of action of PAESe in vivo. However, as is true for other peripherally acting pharmacological agents, the CNS permeability of PAESe is a significant concern, since undesirable side effects can often result from CNS penetration. Moreover, it is highly desirable from

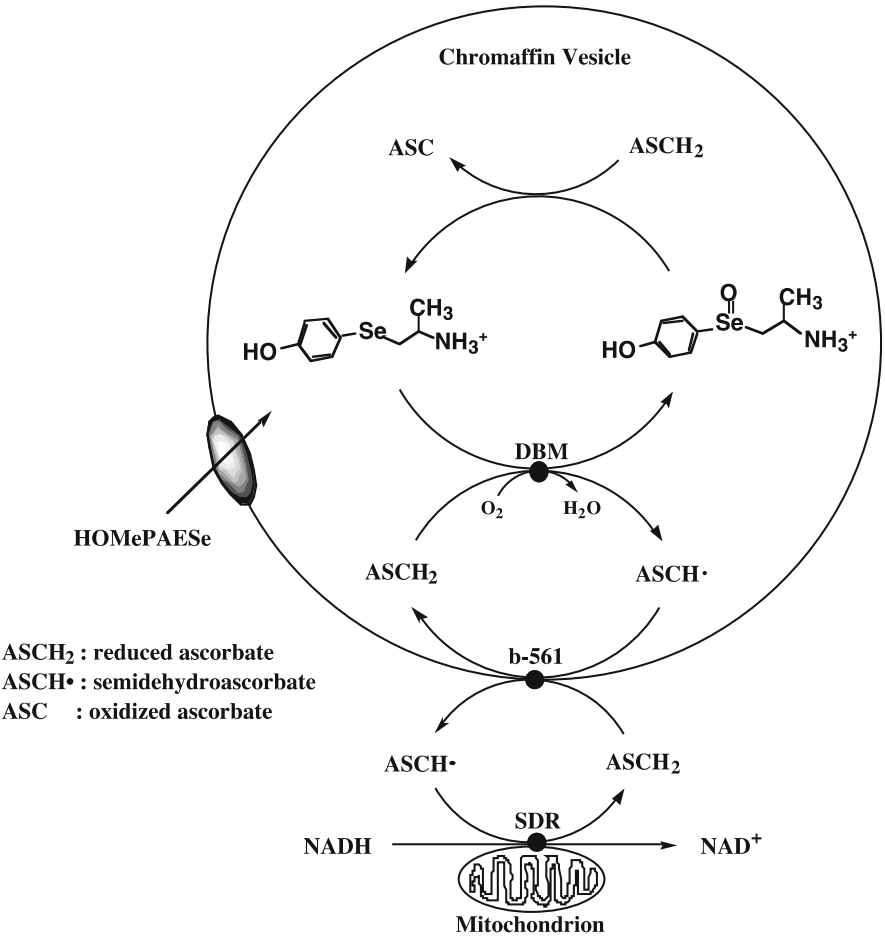


Fig. 1 Dopamine β-monooxygenase-catalyzed selenoxidation of 4-hydroxyphenyl-2-methyl-2-aminoethyl selenide (HOMePAESe), followed by nonenzymatic recycling of the selenoxide product. This results in the *local* depletion of reduced ascorbate within the chromaffin vesicle even in the presence of the b-561-dependent ascorbate recycling system, since the native b561 system can only recycle the semidehydroascorbate generated during DBM turnover but not the fully oxidized ascorbate produced by the nonenzymatic selenoxide/ascorbate reaction

a therapeutic perspective that pharmacological agents used to treat chronic diseases such as hypertension be orally active. We therefore proceeded to develop a PAESe derivative that would exhibit both restricted CNS permeability and oral antihypertensive activity [32].

Briefly, our approach was as follows: First, we employed inductively coupled plasma/mass spectroscopic (ICP/MS) analysis of plasma samples to determine the pharmacokinetic parameters for our selenide compounds. Next, an oxidative procedure for the digestion and processing of tissue samples was then developed in

order to obtain ICP/MS data on the tissue distributions of selenium-containing metabolites following the administration of selenide compounds. The results clearly demonstrated that the aromatic-ring hydroxylation of the selenides results in a marked reduction in brain levels of selenium-containing metabolites. We also demonstrated that α-methylation abolishes the activity of the enzyme MAO toward these compounds and markedly enhances their oral activity; since the intestinal mucosa possesses a considerable amount of MAO, resistance to MAO-catalyzed degradation is an essential characteristic for drugs to exhibit good oral bioavailability. Finally, we investigated the comparative effects of PAESe derivatives on locomotor activity and operant behavior. The results fully corroborated our analytical data, thus confirming the pharmacological relevance of the ICP/MS results and providing a compelling basis for drug design. On the basis of these biochemical, pharmacological, inductively coupled plasma/mass spectrometry, and behavioral studies, we successfully demonstrated that the novel compound (*S*)-4-hydroxyphenyl-2-methyl-2-aminoethyl selenide (HOMePAESe) exhibits both restricted CNS permeability and oral antihypertensive activity [32]. Indeed, this compound is the first orally active selenium-based antihypertensive agent ever reported (Fig. 2).

We carried out a series of experiments designed to probe the hemodynamic mechanism of action of these phenylaminoethyl selenide antihypertensives [33]. In these experiments, a noninvasive pulsed Doppler ultrasound probe was used to measure peak blood flow velocity in the aortic arch from the right second intercostal space. PAESe was found to increase peak aortic blood flow velocity (+44%), heart rate (+16%), and blood flow acceleration (+105%), while decreasing left ventricular ejection time (LVET) (−37%) concomitant with a decrease in mean arterial pressure (−54%). These results were compared with the known vasodilator, hydralazine, which had similar effects on mean arterial pressure (MAP) and peak velocity but caused an increase in LVET (+42%) and a decrease in heart rate (−18%). Taken together, these results indicate that PAESe decreases blood pressure via a decrease in peripheral resistance, which overcomes an initial increase in heart rate and acceleration to give a net decrease in mean arterial pressure.

More recent work in the author's laboratory has focused on elucidating and harnessing the redox cycling and antioxidant activity of phenylaminoethyl selenides [34–37]. We established that PAESe reacts rapidly with many cellular

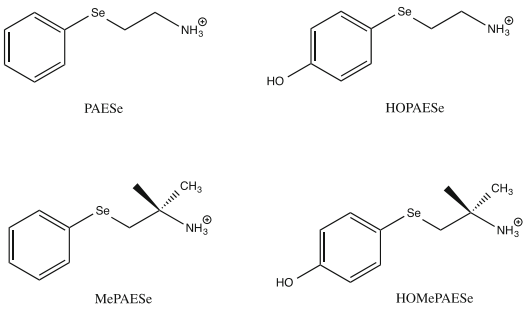


Fig. 2 Structures of phenylaminoethyl selenides investigated in the author's laboratory. It is noteworthy that the compound (*S*)-4-hydroxyphenyl-2-methyl-2-aminoethyl selenide (HOMePAESe) exhibits both restricted CNS permeability and oral antihypertensive activity

oxidants, with the selenoxide product generated from this antioxidant activity being readily recycled back to the selenide by cellular reductants such as ascorbate and glutathione with no complex side reactions. One of the most predominant of such cellular oxidants is peroxynitrite, a powerful oxidizing and nitrating agent formed from a diffusion-controlled reaction between nitric oxide and superoxide in endothelial cells, macrophages, and neutrophils. Reactions of peroxynitrite with cellular components have been linked to oxidative stress and inflammatory tissue damage, as, for example, in atherosclerosis and ischemia–reperfusion injury. Peroxynitrite also reacts readily with DNA where it causes base modification and induction of double- and single-strand breaks. We showed that phenylaminoalkyl selenides protect plasmid DNA from peroxynitrite-mediated damage by scavenging this powerful cellular oxidant and forming phenylaminoethyl selenoxides as the sole selenium-containing product. Moreover, on the basis of kinetic studies, potentiometric titrations, cyclic voltammetry, and MatLab simulations, we demonstrated that glutathione-based selenoxide redox cycling enhances these protective effects of the selenides against peroxynitrite-induced DNA damage.

The above results provide support for the idea that exogenously supplied or metabolically generated organoselenium compounds, capable of propagating a selenium redox cycle, might supplement natural cellular defenses against the oxidizing agents generated during metabolism. While several such organoselenium compounds are under active investigation as potential therapeutic agents, the chemical characterization of reaction intermediates involved in selenium redox cycling has been problematical. Among the proposed selenium intermediates in the redox reactions of organoselenium compounds are species such as selenenic and seleninic acids, selenones, spirodioxaselenanonanes, and thiolseleninates. Thus, for example, evidence has been reported for the formation of a thiolseleninate intermediate in the reactions between ebselen oxide and certain thiols [38, 39] and for a spirodioxaselenanonane intermediate in the reaction between di(3-hydroxypropyl) selenide and *tert*-butyl hydroperoxide [40]. The reactivity and instability of some tetra-coordinate selenium compounds, coupled with fast reaction rates for the reactions of organoselenium compounds with oxidants or with reducing agents such as thiols, have made it difficult for several investigators to clearly characterize thioselenurane-like intermediates (see Cowan et al. [36] for further discussion of this point).

Work in the author's laboratory has provided direct evidence that the reaction between phenylaminoalkyl selenoxides and glutathione (GSH) proceeds through the intermediacy of a thioselenurane species [36]. The results of stopped-flow kinetic experiments were consistent with a rapid and stoichiometric initial reaction of GSH with selenoxide to generate a kinetically detectable intermediate, followed by a slower reaction of this intermediate with a second molecule of GSH to produce the final selenide and GSSG products. Flow injection ESI-MS and ESI-MS/MS experiments confirmed that the reaction intermediate is indeed a thioselenurane. Final structural characterization of the thioselenurane intermediate was obtained from the analysis of the daughter ions produced in flow injection ESI-MS/MS experiments. These results elucidate the chemical nature of the redox cycling of

phenylaminoalkyl selenides and represent, to our knowledge, the first evidence for the intermediacy of a thioselenurane species in the reaction of thiols with selenoxides. Previously, Kumar et al. [38] had proposed the formation of a thioselenurane in the reaction between benzenethiol and a substituted phenyl benzyl selenoxide, since they observed via ESI-MS a cyclization product that could arise from such a species; however, they were unable to obtain any structural evidence for this proposed intermediate (Fig. 3).

Hydrogen peroxide, produced in living cells by oxidases and by other biochemical reactions, plays an important role in cellular processes such as signaling and cell cycle progression [41, 42]. Nevertheless, hydrogen peroxide is capable of inducing damage to cellular components, and it can be converted through Fenton chemistry to even more reactive molecules, such as hydroxyl and peroxide radicals. Indeed, oxidative stress – the cellular state arising from overproduction of hydrogen peroxide and other reactive oxygen species (ROS) – has been linked to cellular pathologies ranging from DNA and cellular membrane damage to more complex disorders such as inflammatory diseases and cancer [43]. In experiments carried out in the author's laboratory [37], we made use of peroxalate nanoparticle methodology to achieve chemiluminescent imaging of hydrogen peroxide consumption by phenylaminoethyl selenides. Further, we demonstrated that phenylaminoethyl selenides decrease lipopolysaccharide (LPS)-induced oxidative stress in human

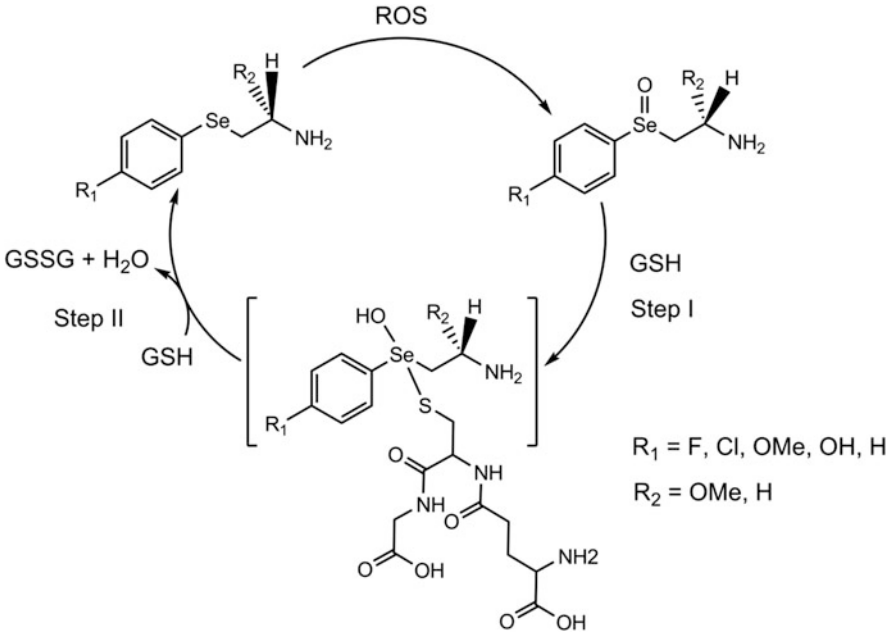


Fig. 3 Identification of a thioselenurane intermediate in the reaction between phenylaminoalkyl selenoxides and glutathione

embryonic kidney cells. We also encapsulated PAESe within poly(lactide-co-glycolide) nanoparticles, and we showed that these selenide-loaded nanoparticles exhibit antioxidant activity in a mammalian cell line. Taken together, these results significantly enhance the attractiveness of phenylaminoethyl selenides as potential agents for supplementing cellular defenses against reactive oxygen species.

Anthracyclines such as doxorubicin (DOX) and daunorubicin are widely used anticancer agents that are effective in the treatment of acute leukemia, non-Hodgkin's lymphomas, and breast, ovarian, and lung cancers [44]. However, the clinical use of anthracycline is limited by severe dose-limiting cardiotoxicities, such as cardiomyopathy and congestive heart failure. This cardiotoxicity is now generally believed to result primarily from the generation of reactive free radicals such as superoxide anion, hydroxyl radical, and peroxynitrite [45, 46]. Dexrazoxane, a potent iron chelator that interrupts anthracycline-iron-mediated free radical generation, is in clinical use to decrease this free radical-associated toxicity of DOX [47, 48]. However, dexrazoxane is known to cause myelosuppression, and recent studies suggest that its use may lead to acute myeloid leukemia and myelodysplastic syndrome. The FDA has noted (http://www.fda.gov/Drugs/DrugSafety/ucm263729.htm) that the European Medicines Agency has announced restrictions on dexrazoxane usage to certain types of patients [48].

In collaboration with the laboratory of Dr. Robert Arnold, we have carried out a series of studies [49] aimed at determining the potential of PAESe to mitigate anthracycline-induced cardiotoxicity. First, we determined the effects on the growth of human prostate carcinoma (PC-3) cells of combined administration of PAESe with DOX, vincristine, or *tert*-butyl hydroperoxide (TBHP). DOX and vincristine are, of course, used clinically as anticancer drugs, whereas *tert*-butylhydroperoxide is a potent oxidizing agent known to exert oxidative-mediated cytotoxicity on PC-3 cells. We found that while PAESe had little effect on the activities of DOX or vincristine, the selenide significantly decreased the cytotoxic effect of TBHP in a dose-dependent manner. Moreover, using the cell-permeable indicator, CM-H_2DCFDA, we showed that PAESe decreased the formation of intracellular reactive oxygen species from either TBHP or DOX. We then proceeded to determine the effect of PAESe on the antitumor activity of DOX in an in vivo xenograft tumor model of human prostate cancer in NCr nude mice. We found that PAESe did not alter DOX antitumor activity and also showed evidence of direct antitumor activity relative to controls. Most importantly, PAESe decreased DOX-mediated infiltration of neutrophil and macrophages into the myocardium, which are early signs of cardiotoxicity. In addition, recent experiments in H9C2 cells have shown that concomitant treatment with PAESe significantly reduced DOX-mediated expression of ANP and MHC-β, which are markers of cardiac hypertrophy (unpublished observations). These findings suggest that selenides such as PAESe may be quite attractive agents for mitigating the cardiotoxicity associated with clinical use of DOX and related anticancer drugs.

3 Ebselen and BXT-51072

Ebselen (2-phenyl-1,2-benzisoselinazol-3[2H]-one) is an organoselenium compound that has been shown to mediate the reduction of hydroperoxides by thiol compounds such as glutathione, thereby modeling the enzymatic activity of the selenoenzyme, glutathione peroxidase [50]. Many researchers have carried out a number of chemical, biochemical, mechanistic, and pharmacological studies on ebselen and its chemical analogs, and a commentary providing historical perspective on the early research and development of ebselen has recently been published [51]. Over the years, a key rationale underlying much of this interest in ebselen has been the possibility that selenium redox cycling in ebselen or its derivatives might supplement natural cellular defenses against oxidizing agents. However, it has now become quite clear that the redox cycle of ebselen consists of multiple reaction pathways with several intermediates and by-products [52–55]. Moreover, several investigations have shown that ebselen is an inefficient catalyst due to deactivating pathways, where unreactive intermediates hinder the regeneration of the original compound [54, 56–58]. In contrast, as noted above, stopped-flow kinetic and ESI-MS/MS results in this author's laboratory have demonstrated that the redox cycling of phenylaminoethyl selenides is a rapid and efficient two-step process, with initial formation of a thioselenurane intermediate followed by complete regeneration of the original selenide (Fig. 4).

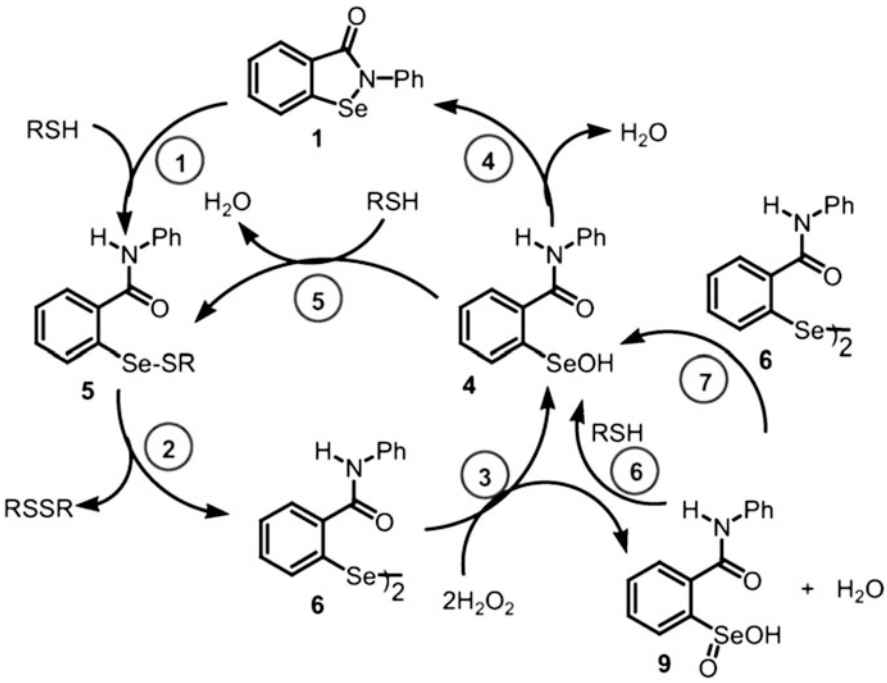
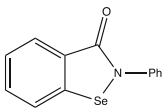


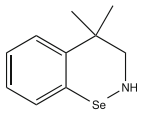
Fig. 4 Mechanism for the GPx activity of ebselen as proposed by Sarma and Mugesh [54]

Several years ago, a clinical trial was undertaken in Japan to assess whether ebselen is capable of protecting against the oxidative tissue damage which occurs following acute ischemic stroke [59]. The results of this trial showed that the most significant improvement was achieved in patients who started a 14-day course of ebselen treatment within 24 h of stroke onset; however, these benefits were statistically significant when evaluated after 1 month but not after 3 months. Subsequently, recruitment was announced for a phase III trial in Japan, sponsored by the Daiichi Pharmaceutical Company, to test the efficacy of ebselen in patients with acute cerebral infarction (cortical infarction). According to the Web site http://www.strokecenter.org/trials/TrialDetail.asp?trialName=list/trialPage298.htm, 394 patients were enrolled in this placebo-controlled, double-blind, randomized, multicenter trial, with enrollment to be completed by November 2002. Unfortunately, results from this trial have never been posted on this Web site, and this author could not find any publications in the open scientific literature reporting results from this trial. In their recent commentary on the early research and development of ebselen [51], Parnham and Sies state that the results of the trial were submitted for registration to the Japanese regulatory authority (MHLW), but the reviewers considered the drug efficacy to be insufficient for approval. According to media reports (see, e.g., http://www.bbsrc.ac.uk/news/health/2013/130114-n-new-drug-bipolar-disorder.aspx), researchers in Oxford have begun a small study in healthy volunteers to test whether ebselen has lithium-like effects on brain function in humans; if successful, these researchers reportedly plan to move on to a small phase II trial in people with bipolar disorder.

Ebselen has been investigated in animal models in a number of laboratories for other possible pharmacological activities. For example, Kono et al. [60] reported that twice-daily treatment of rats with 50 mg/kg ebselen significantly reduces early alcohol-induced liver injury, as measured by liver enzyme assays, inflammation, and liver necrosis. Another example is the report of Moussaoui et al. [61] that ebselen, when administered before, during, and after injections of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), prevents both neuronal loss and clinical symptoms in a primate MPTP model of Parkinson's disease. More recently, Chew et al. [62] studied the antiatherosclerotic and renoprotective effects of ebselen in diabetic apolipoprotein E/GPx1-double knockout mice that had been injected with streptozotocin. They found that that ebselen reduced atherosclerosis and that it also prevented the changes in renal structure and function and the inflammatory responses associated with nephropathy in the diabetic mice. These findings are reminiscent of the earlier work of Chander et al. [63], who reported that ebselen improved renal outcomes in the Zucker diabetic fat rat. Ebselen been reported to protect neuronal cells in the ventroposterior nucleus of stroke-prone renovascular hypertensive rats against damage resulting from a cerebral cortical infarction [64]. Along these lines, Yamagata and coworkers [65] have reported that ebselen exhibits protective effects against neurodegeneration induced by hypoxia and reperfusion in stroke-prone spontaneously hypertensive rats.



Ebselen



BXT-51072

BXT-51072 [4,4-dimethyl-2,3-dihydro-1,2-benzoselenazine], an organoselenium compound that is structurally related to ebselen, is severalfold more reactive than ebselen in catalyzing hydroperoxide reduction by glutathione [66]. A US patent [67] describes the development of BXT-51072 and analogs as potential treatments for inflammatory bowel disease (IBD) and for possible use in treating asthma, chronic obstructive pulmonary disease, and stroke. A successful phase I trial, carried out by Oxis International [68], was followed by an early phase II trial that apparently showed a promising improvement in patients with mild to moderate ulcerative colitis. However, in a subsequent form 10QSB filing with the US Securities and Exchange Commission [69], Oxis made the following statement:

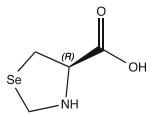
> "We have granted a licensee exclusive worldwide rights, in certain defined areas of cardiovascular indications, to develop, manufacture and market BXT-51072 and related compounds from our library of such antioxidant compounds. The licensee is responsible for worldwide product development programs with respect to licensed compounds. Due to the lack of financial resources, we ceased further testing of BXT-51072 but continue to review the possibility of further developing applications for BXT-51072 and related compounds outside of the areas defined in the license. However, further development and commercialization of antioxidant therapeutic technologies, oxidative stress assays or currently unidentified opportunities may require additional capital."..."No assurances can be given that we will be able to raise such funds in the future on terms favorable to us, or at all."

In January 2004, Axonyx Inc. acquired approximately 53% of the outstanding voting stock of Oxis International.

4 Selenazolidine Prodrugs

Roberts and coworkers [70, 71] have made the argument that inorganic forms of selenium have a relatively narrow therapeutic index, and since their metabolic conversion to H_2Se depletes glutathione, they may exhibit unacceptable toxicity for long-term or high-dose administration in humans. Moreover, these investigators pointed out that while the organoselenium compound, selenomethionine (often present in nutritional supplements as a component of "selenized" yeast), is able to provide selenium to correct nutritional deficiency, one of its major metabolic fates is nonspecific incorporation into proteins in place of methionine, which thereby decreases the therapeutic availability of the selenium by sequestering it inappropriately in protein. The metabolism of selenocysteine, on the other hand, does give rise to an increase in the selenium pool leading to selenoproteins as well as to formation of methylated metabolites; both of these effects can contribute to selenium-based anticancer activity. However, selenocysteine is chemically unstable since it is easily oxidized, and it is therefore relatively difficult to handle.

Based on these considerations, Roberts and coworkers [70–72] pioneered the development of selenazolidine-4(*R*)-carboxylic acids as prodrug forms of selenocysteine for potential use in therapeutic applications.



selenazolidine-4(R)-carboxylic acid

These prodrugs are condensation products of selenocysteine and carbonyl compounds, and contain selenazolidine rings with various substituents; they are designed to release selenocysteine either enzymatically or through spontaneous hydrolysis. The selenazolidines were found to be much less toxic than sodium selenite in cultured lung fibroblast cells; this is as expected since organoselenium compounds have generally been perceived as being less toxic than inorganic selenium species. Treatment of these cells with selenazolidines increased cellular glutathione peroxidase activity, and a clear stereochemical preference was observed, with the prodrugs of L-selenocysteine being more active than the D-enantiomers in increasing glutathione peroxidase activity. Overall, the selenazolidines exhibited comparable chemopreventive activity to selenocysteine, with decreased cytotoxicity and greater biological selenium availability than sodium selenite or selenomethionine.

As discussed below, many cancers are known to overexpress thioredoxin reductase, and knockdown of thioredoxin reductase can enhance the sensitivity of cancer cells to anticancer agents. Accordingly, Poerschke and Moos [73] extended the earlier work of Roberts by utilizing a lentiviral microRNA delivery system to knockdown thioredoxin reductase expression in human lung adenocarcinoma cells and then examining the cytotoxic activities of the selenazolidine prodrugs, 2-butylselenazolidine-4(*R*)-carboxylic acid and 2-cyclohexylselenazolidine-4-(*R*)-carboxylic acid. They found that thioredoxin reductase knockdown increased the cytotoxicity of selenazolidines in these adenocarcinoma cells via a mechanism involving mitochondrial dysfunction and caspase-independent activation of the apoptosis-inducing factor.

5 Selenium-Carrier Conjugates

In a series of US patents [74–76], Spallholz and Reid described the methodology for preparing selenium-carrier conjugates which could then be specifically targeted for delivery to tumors or to sites of pathogenic infection. The underlying rationale of this methodology is the idea of utilizing specific organoselenium compounds to

generate superoxide and other free radicals in a localized environment. Selenium Ltd. (http://www.selenbio.com), a company established in 2004 based on the work of these investigators, claims to have developed the necessary technology for covalently attaching selenium-containing molecules to antibodies, peptides, polymers, and drugs. Thus, for example, selenium-modified humanized antibodies and proteins have been used to effectively kill human prostate, colorectal, and lymphatic cancer cells in tissue culture, with the operative mechanism being the generation of superoxide by the antibody-linked organoselenium compound at the target site which induces apoptosis and destroys the tumor cell. Similarly, an organoselenium-labeled CD-4 peptide directed against the HIV-1 virus destroyed the virus before it was able to infect T cells, with 95% of a virulent isolate of HIV being inactivated in 2 h.

Another potential use for selenium-carrier conjugates is in the treatment of infections caused by bacteria, especially in a biofilm. Tran et al. [77] examined the ability of an organoselenium–methacrylate polymer (Se-MAP) to block biofilm formation by both *Pseudomonas aeruginosa* and *Staphylococcus aureus*. These bacteria were chosen since they cause a major share of wound infections and because drug-resistant forms of these bacteria have become a serious problem in the treatment and management of such infections. The results showed that 0.2% (wt/wt) Se in Se-MAP, covalently attached to cellulose disks, completely inhibited *P. aeruginosa* and *S. aureus* biofilm formation. The authors surmise that inhibition of biofilm formation is due to damage to bacterial cell walls and DNA by superoxide, which is generated via selenium-mediated thiol oxidation. The authors also found that the Se-MAP coating is stable and not cytotoxic to mammalian cells. Thus, they suggest that the application of Se-MAP-coated gauze to the debrided tissues of burn wounds may prevent the development of biofilms and facilitate wound healing. This approach might also be used for dental applications [78] and to combat infections in hospitals caused by catheters and other medical implants.

Kunstelj et al. [79] have patented the idea of producing a water-soluble polymer possessing a reactive selenium group (e.g., PEG coupled to selenocysteine or selenocystamine). The polymer is then reacted with a pharmaceutically active agent such as granulocyte colony-stimulating factor (G-CSF) to form a conjugate through linkage of the polymer's Se moiety to a Cys of the pharmaceutically active agent via an –Se–S– bond. The inventors visualize the use of this type of conjugate for the treatment of diseases such as neutropenia. Miki et al. [80] have patented an approach for inhibiting tumor cell growth that entails the administration of a selenium-containing prodrug while also administrating directly to the tumor an expression system for an enzyme for which the prodrug is a substrate. Thus, for example, SeMet is administered together with a vector for expression of methionine lyase, such that the vector preferentially replicates in rapidly proliferating cells or is under control of a promoter that is operable selectively in tumor tissue, thus conferring specificity for tumor cells. The lyase cleaves the prodrug at the C–Se bond, thus liberating a toxic form of selenium (presumably RSe^-) at the site of the tumor in an amount sufficient to inhibit tumor cell growth.

6 Targeting the Thioredoxin–Thioredoxin Reductase System

A number of investigators have been pursuing the development of compounds that target the mammalian selenoenzyme, thioredoxin reductase (TrxR), which catalyzes the NADPH-dependent reduction of the redox protein, thioredoxin (Trx). The Trx/TrxR system functions as a donor of reducing equivalents for enzymes such as ribonucleotide reductase, which is essential for DNA synthesis, and protein disulfide reductase, which is critically involved in thiol/disulfide redox regulation (Fig. 5). Therefore, the inhibition of TrxR would be expected to be highly detrimental to the growth of tumor cells, especially since the levels of both TrxR and Trx have been found to be elevated in tumor cell lines, and expression profiles of thioredoxin family proteins correlate with cell proliferation, survival, and tumor grade [81–84]. Moreover, knockdown of thioredoxin reductase in murine lung carcinoma cells reversed the malignant phenotype and decreased tumor growth and metastasis [85].

As mentioned above, Poerschke and Moos [73] have reported that knockdown of thioredoxin reductase in human lung adenocarcinoma cells using a lentiviral microRNA delivery system increased the cytotoxicity of selenazolidines in these adenocarcinoma cells. In earlier work, Powis and coworkers [86] found that certain organoselenium and organotellurium compounds, primarily of the diaryl chalcogenide type, were inhibitors of TrxR and also growth inhibitors of cultured tumor cells; these compounds, with one exception, did not inhibit glutathione reductase. Subsequently, these investigators reported [87] that water-soluble organotellurium

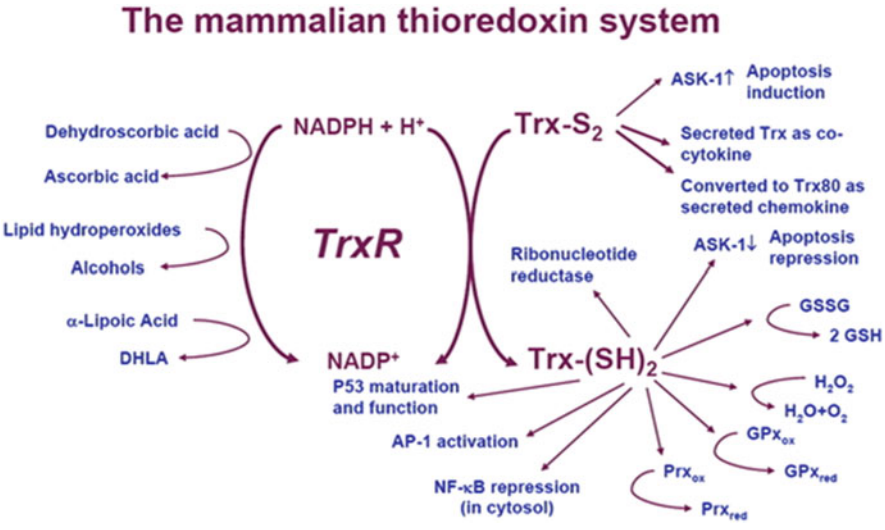


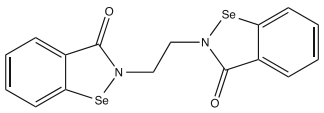
Fig. 5 The mammalian thioredoxin–thioredoxin reductase (Trx/TrxR) system is linked to many cellular processes (from http://biochem.mbb.ki.se/Arner/research/img/Trx_TrxR.jpg)

compounds of the diaryl telluride, alkyl aryl telluride, and dialkyl telluride types are very potent inhibitors of TrxR, and these tellurides inhibited the growth of MCF-7 and HT-29 human cancer cells in culture at the 5–10 micromolar level; however, their hydrophilicity did seem to restrict cellular uptake.

In 2002, Holmgren and coworkers reported [88] that ebselen is an excellent substrate for human TrxR. They showed that TrxR, in the presence of thioredoxin, catalyzes a very rapid reduction of ebselen to ebselen selenol, with NADPH serving as the electron donor. The reduced ebselen can, in turn, react rapidly with hydrogen peroxide (peroxidase activity), thus setting up a catalytic cycle whereby hydrogen peroxide and lipid peroxides can be eliminated. Since TrxR and Trx are present in all cells throughout the body, the authors proposed that this peroxidase activity provides a mechanistic explanation for the antioxidant and anti-inflammatory effects of ebselen and, in particular, for its activity in protecting cells against ischemic tissue damage. They further stated that their results "demonstrate that the mechanism of action of ebselen may be predominantly via the thioredoxin system rather than via glutathione." Recently, Lu and Holmgren [89] have pointed out that the absence of a glutathione-based antioxidant system in pathogenic bacteria such as *Helicobacter pylori*, *Mycobacterium tuberculosis*, and *S. aureus* makes the bacterial thioredoxin-based antioxidant system essential for the survival of such organisms under conditions of oxidative stress. They therefore conclude that the inhibition of TrxR accounts for ebselen's antibacterial activity in such organisms.

This issue of the relationship between the thioredoxin-based and glutathione-based antioxidant systems, and the possibility of cross talk between these systems, is very relevant to the findings of Casagrande et al. [90], who showed that Trx undergoes glutathionylation in human T cell blasts that had been exposed to oxidative stress. Glutathionylation, which was shown by MALDI-TOF mass spectrometry to occur on rhTrx's Cys72 residue, abolished the ability of Trx to act as a disulfide reductase in the presence of TrxR and NADPH. The authors conclude that their finding of Trx regulation by glutathionylation indicates that cross talk exists between the thioredoxin and the glutathione systems.

With the recognition that many tumor cells exhibit a high level of expression of Trx and TrxR, which renders such cells more resistant to apoptosis and chemotherapy, there is obviously considerable interest in the clinical evaluation of TrxR inhibitors as potential anticancer agents. Ethaselen (1,2-[bis(1,2-benzisoselenazolone-3(2H)-ketone)]ethane), a novel organoselenium compound developed by Zeng and coworkers [91], is the first selenium-containing inhibitor of mammalian TrxR1.



Ethaselen

Ethaselen specifically targets the catalytically essential cysteine–selenocysteine (Cys–Sec) redox pair at the C-terminal active site of mammalian TrxR thereby

inhibiting the enzyme, and this leads to an accumulation of oxidized Trx and increased levels of reactive oxygen species. Ethaselen exhibits anticancer activity with low toxicity in tumor cells and in animal models, and inhibition of cancer cell growth by ethaselen correlates with TrxR1 inactivation in several tumor cell lines. As of June 2014, recruitment was underway in China for a phase 1c single-arm study of ethaselen for the treatment of non-small cell lung cancer in patients who had previously received more than two lines of standard treatment (clinicaltrials.gov identifier: NCT02166242). Reportedly, ethaselen has now entered phase II clinical trials – presumably in China – targeting gastric, lung, and colon cancers (see Li et al. [92]).

It should be noted that a number of non-selenium-based anticancer drugs undergoing clinical evaluation are thought to target the Trx/TrxR system. Among these are PX-12 (1-methylpropyl 2-imidazolyl disulfide) for patients with advanced gastrointestinal cancers (phase Ib) [93] and motexafin gadolinium, in combination with doxorubicin (phase I) for patients with advanced solid tumors [94] and in combination with pemetrexed (phase II) for second-line treatment of patients with non-small cell lung cancer [95]. In addition, a number of well-established chemotherapeutic agents are known inhibitors of the Trx system (see Mahmood et al. [96] and references therein).

7 Selenium-Containing Metabolites

Several years ago, Combs [97, 98] pointed out that a number of selenium compounds had been found to be antitumorigenic in a variety of animal models at intakes that are substantially greater than those associated with maximal expression of the known selenocysteine-containing enzymes. This supports the view that the antitumorigenic effects seen in selenium supplementation studies arise at least in part from enhanced production of specific Se-containing metabolites, not just from maximal expression of selenoenzymes. Combs proposed a two-stage model in which selenium supplementation of individuals with low, nutritionally deficient, natural intakes of this element enhances the activities of protective selenoenzymes, whereas selenium supplementation of individuals who are not selenium deficient results in the beneficial buildup of antitumorigenic selenium metabolites. At about the same time, Ganther [99] reemphasized that alterations in selenoenzyme activity had failed to explain the anticancer effects of selenium, and therefore mechanisms involving low-molecular-weight selenium metabolites must also be involved. In subsequent years, a number of low-molecular-weight selenium-containing compounds known – or, quite often, simply postulated – to be formed in the course of selenium metabolism have been studied for possible therapeutic potential (see [100, 101] for recent reviews).

In this author's view, it stands to reason that the specific metabolic state of a particular cell, tissue, or organ should sensitively affect the bioactivity of a given selenium supplement or metabolite. Thus, for example, as recently mentioned by

Zeng and coworkers [91], the well-studied chemopreventive selenium compounds selenite, selenodiglutathione, and methylseleninate are all substrates of mammalian TrxR1, and the product selenium metabolites formed from these compounds play important roles in selenium-induced cytotoxicity and apoptosis in cancer cells. Therefore, the degree to which TrxR1 activity is upregulated or downregulated in a given metabolic situation or disease state would be expected to have a very significant effect on the steady-state levels of these bioactive product metabolites. In this regard, Weekley and Harris recently published [101] an excellent comprehensive review of the current evidence for the various metabolic pathways of common dietary selenium compounds, such as selenite, selenomethionine, methylselenocysteine, and selenocysteine. They conclude that dietary selenium compounds should be considered prodrugs, whose biological activity depends on the activity of the various metabolic pathways in, and the redox status of, cells and tissues. Obviously, such factors will need to be considered carefully when selecting selenium compounds for future trials of disease prevention and treatment by selenium supplementation.

As stated at the outset, a full discussion of the numerous studies on nutritional and epidemiological aspects of selenium metabolites and supplements is beyond the scope of this review. Nevertheless, a few illustrative examples will be mentioned here (Fig. 6).

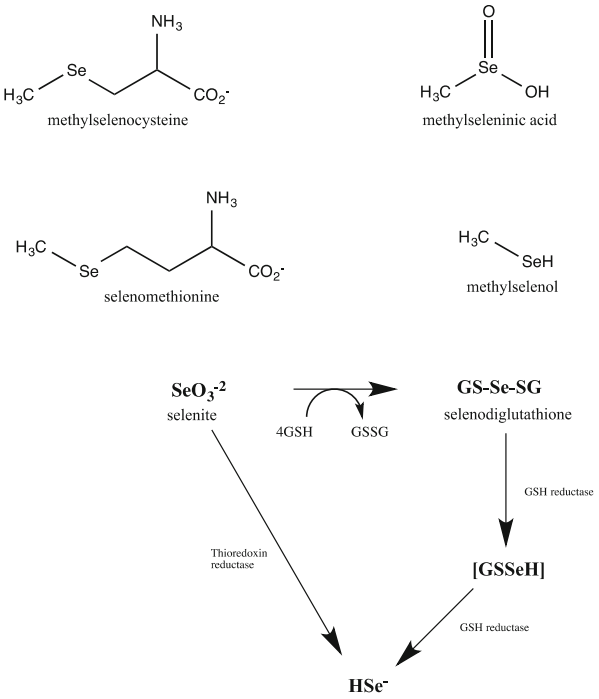


Fig. 6 Structures of some selenium metabolites

The initial step in metabolism of inorganic selenite (SeO_3^{2-}) is its reaction with glutathione to form selenodiglutathione (GS-Se-SG), which is subsequently reduced enzymatically to hydrogen selenide (HSe^-). Selenodiglutathione has been extensively studied by many investigators and shown to be a potent carcinostatic agent in animals and an inhibitor of cell growth and inducer of apoptosis in tumor cells [102–104]. Spyrou et al. [105] have shown that both selenodiglutathione and selenite inhibit the binding of the transcription factor AP-1 to DNA nuclear extracts of 3B6 lymphocytes. Selenodiglutathione is also a facile substrate for the thioredoxin reductase system and glutathione reductase [106] and was found to inhibit human thioredoxin by oxidation of structural thiol groups [107]. It should be noted that there are numerous published studies reporting anticancer effects of inorganic selenite itself, and it is possible that some chemoresistant malignant cells may exhibit enhanced sensitivity to selenite [108]. One promising study in *human patients* was reported by Asfour et al. [109], who found that administering high doses of sodium selenite along with standard chemotherapy increased apoptosis of lymphoma cells in adult patients with non-Hodgkin's lymphoma.

The methylated selenium metabolites methylselenocysteine and methylseleninic acid inhibit the progression and metastasis of cancer and increase survival in the transgenic adenocarcinoma of prostate mouse model [110]. Methylseleninic acid reduces the spontaneous metastasis of Lewis lung carcinoma in mice [111] and also increases the apoptosis potencies of the topoisomerase I inhibitor 7-ethyl-10-hydroxycamptothecin, the topoisomerase II inhibitor etoposide, or the microtubule inhibitor paclitaxel/taxol, in cell lines of advanced human metastatic prostate cancer against which standard chemotherapeutic treatments have limited efficacy [112]. Methylselenocysteine, which is found in foods such as garlic, onions, and broccoli, has been shown in combination with tamoxifen to inhibit MCF-7 breast cancer xenografts in nude mice through elevated apoptosis and reduced angiogenesis [113]. Methylselenocysteine and methylseleninic acid are cleaved metabolically to produce methylselenol ($MeSe^-$); Ganther and coworkers [114] have shown that the production of this monomethylated selenium metabolite is a key step in cancer chemoprevention by compounds such as methylselenocysteine and methylseleninic acid. Methylselenocysteine has recently been called "a promising antiangiogenic agent for overcoming drug delivery barriers in solid malignancies for therapeutic synergy with anticancer drugs" [115].

Selenomethionine is the form of selenium used in the National Cancer Institute's SELECT trial and in a number of other selenium supplementation studies. Selenomethionine is capable of binding to methionine t-RNA and becoming incorporated into proteins in place of methionine [116]. Selenomethionine at micromolar concentration was found to inhibit growth of three human tumor cell lines, whereas growth inhibition of normal diploid fibroblasts required millimolar concentrations [117]. Thus, at least in these cell lines, cancer cells apparently exhibit greatly enhanced sensitivity to the growth inhibitory effects of selenomethionine. Mice treated orally or topically with selenomethionine were found to have significantly lowered UV irradiation-induced skin damage and reduced incidence of skin cancer

[118]. Frenkel and Caffrey [119] have reported that two selenium compounds, selenite and selenomethionine, are able to reduce the induction of resistance by ovarian cancer cells or xenografts to the chemotherapeutic agents melphalan or cisplatin. Similarly, selenomethionine and selenite were found to prevent the induction of resistance to the antitumor agent cisplatin in mice bearing tumors derived from human ovarian cells [120]. These are interesting findings, since the development of drug resistance is considered to be a major cause for the failure of chemotherapy in several types of cancer.

A clever approach to selenium-based gene-directed enzyme prodrug therapy for cancer was devised by Miki et al. [121]. Their strategy exploits the toxic prooxidant property of methylselenol, which is released from selenomethionine by cancer cells that had been transduced with the adenoviral-delivered methionine α,γ-lyase gene. In these cells, the cytotoxicity of the selenomethionine prodrug was increased up to 1,000-fold compared with nontransduced cells. A strong bystander effect occurred due to methylselenol release from the transduced cells and uptake by surrounding tumor cells. Methylselenol damaged the mitochondria via oxidative stress and caused cytochrome c release into the cytosol, thereby activating the caspase cascade and apoptosis. These investigators also reported that treatment using their prodrug approach inhibited tumor growth in rodents and significantly prolonged their survival.

8 Diaryl Diselenides

In a series of nutritional and behavioral studies, diphenyl diselenide compounds were found to improve memory in a rodent model of Alzheimer's-type sporadic dementia, thereby ameliorating learning performance in these animals [122]. In subsequent studies, these investigators investigated the molecular mechanism of neuroprotection by *p*,*p*′-methoxyl-diphenyl diselenide in cortical neurons exposed to amyloid-β (Aβ) peptide as well as in Aβ-infused mice [123]. The diselenide compound was found to prevent Aβ-induced cell death associated with the inhibition of caspase-3 and caspase-9 activities, poly(ADP-ribose) polymerase cleavage, and JNK activation. In addition, oral administration of the diselenide at 5 mg/kg for 5 days rescued memory impairment in mice that had been exposed to Aβ fragment via intracerebroventricular infusion. Taken together, these findings are both timely and noteworthy, given the intense current interest in developing new approaches for the treatment of Alzheimer's disease.

Diaryl diselenides have also been investigated as potential anti-inflammatory agents. Shin et al. [124] reported that bis-(3-hydroxyphenyl) diselenide inhibits LPS-stimulated iNOS and COX-2 expression in RAW 264.7 macrophages. The diselenide compound also significantly reduced the release of TNF-α, interleukin (IL)-1β, and IL-6, and the authors provided evidence that these activities are due to the downregulation of NF-κB binding activity. In addition, disubstituted diaryl diselenides have been found to reduce carrageenan-induced paw edema in rats

[100], and there is a report [125] that diphenyl diselenide produces a significant peripheral antinociceptive (i.e., analgesic) effect via a mechanism that is unlike the activation of opioid, dopaminergic D2, or muscarinic cholinergic receptors.

It should be noted that in a recent review article, Nogueira and Rocha [126] refer to diphenyl diselenides as "Janus-faced" molecules, since the interaction of diphenyl diselenide with thiols can give rise to either toxic or beneficial effects. In truth, this appellation could apply as well to a number of other organoselenium compounds (see below).

9 Selenium Versus Sulfur in Drug Design

Although selenium and sulfur are members of the same periodic table group, there are important differences between the chemical properties of these two elements (see Iwaoka and Arai [127] and references therein for a detailed comparison). Selenium has a lower electronegativity and larger atomic radius than sulfur. Bond dissociation energies for C–Se, Se–H, and Se–Se covalent bonds are 58, 67, and 44 kcal/mol, respectively, whereas the corresponding values for sulfur are 65, 88, and 63 kcal/mol. Thus, organoselenium compounds should be more reactive than organosulfur compounds; selenols are more reactive than thiols and Se–Se bonds can be more easily cleaved than S–S bonds. Importantly, the ability of selenoxides to act as mild oxidizing agents has been recognized for decades [128].

The differences between selenium and sulfur can be very significant from the perspective of drug design. One illustrative example is provided by work carried out in this author's laboratory on the markedly differing effects of selenide versus sulfide compounds on norepinephrine production in adrenal chromaffin granules [26–29]. Both phenylaminoethyl selenide and its sulfur cognate, phenylaminoethyl sulfide, are readily taken up into chromaffin granules, where they undergo facile DBM-catalyzed heteroatom oxygenation to produce the corresponding selenide or sulfoxide product, respectively. However, only the selenoxide product has the ability to propagate a turnover-dependent redox cycle that results in local depletion of reduced ascorbate within the chromaffin vesicle. In contrast, the sulfoxide product formed enzymatically from phenylaminoalkyl sulfide is not capable of propagating such a redox cycle, and depletion of reduced ascorbate in the chromaffin granule consequently does not occur with the sulfide compound. We demonstrated [34] on the basis of potentiometric titrations that these findings are fully consistent with the redox potentials of the phenylaminoethyl selenoxides, which range from +410 to +480 mV (depending on the ring substituents) at the pH of the chromaffin granule milieu. Cyclic voltammetry experiments confirmed that the peak potentials for the reductive waves, E*pc*, for sulfoxides are ca. 500 mV lower than the E*pc* for the corresponding phenylaminoethyl selenoxides.

More than two decades ago, Ip and Ganther [104] compared the cancer chemopreventive efficacy in a rat mammary tumor model of three pairs of selenium and sulfur analogs; the compounds tested were selenocystamine/cysteamine,

Se-methylselenocysteine/S-methylcysteine, and selenobetaine/sulfobetaine. In all cases, the selenium compounds were found to be far more active in chemoprevention than their structurally similar sulfur cognates, and the authors concluded that selenium compounds may both prevent cellular transformation and delay or inhibit the expression of malignancy after carcinogen exposure. More recently, Xiao and Parkin [129] investigated the effects of 27 selenium compounds and 16 structurally related organosulfur compounds on phase II enzyme activity induction (a chemopreventive mechanism) in murine hepatoma cells, and identified nine highly active species, all of which were organoselenium compounds (Fig. 7).

The issue of the relative activities of organoselenium versus organosulfur compounds as antioxidants has been discussed by a number of investigators. For example, Steinmann et al. [130] found that the reactions of selenocysteine and selenoglutathione with tyrosyl radicals in *N*-Ac-Tyr-NH_2 and in insulin proceed orders of magnitude more rapidly than the reactions of the corresponding sulfur analogs. These authors point out that the effective concentration of a selenocysteine residue in the microenvironment of a selenoprotein is much higher than its concentration in the aqueous compartments of the cell, so a protective function that entails repair of radical damage to neighboring residues can be envisioned for selenocysteine. Similarly, phenylaminoethyl selenides have been found to be much more effective in protecting pUC19 DNA against peroxynitrite-induced damage than the corresponding sulfur analogs [34]. Iwaoka and Arai [127] have expressed the view that "progress in selenium biology [*has*] allowed researchers in biological chemistry to expand their research arena from sulfur to selenium." While this statement is quite evidently true, there are likely major differences between the mechanisms that underlie the biological activities of organoselenium versus organosulfur compounds, and this must certainly be kept in mind when extrapolating to selenium from the large body of information that has accumulated over the

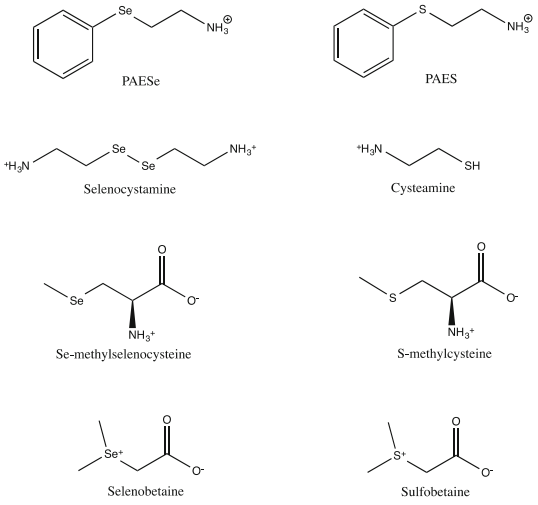


Fig. 7 Selenium compounds and their sulfur analogs

years about the biology of sulfur compounds. It is reasonable to expect that relevant mechanistic details will continue to be further elucidated in future investigations.

10 Conclusions and Perspectives

Selenium has a long history of association with human health and disease. We now recognize that this element is an essential nutrient that is critical to key cellular processes and to the activities of a number of important enzymes. Great strides have been made over the past few years in our understanding of the biochemistry of selenium metabolism, and these efforts have been facilitated by structural determinations of low-concentration metabolites using advanced separation and mass spectral techniques. With regard to selenoproteins, we now know that the selenoproteins constituting the human selenoproteome are encoded by 25 genes in the human genome [13], and very significant progress is being made in characterizing selenoproteins and their physiological functions. As discussed by Labunskyy et al. in their excellent and very recent review [131], selenoproteins not only function as antioxidant enzymes but also in diverse cellular processes such as thyroid hormone function, selenophosphate synthesis, protein folding, and protein degradation. However, the biological functions of approximately half of the human selenoproteins still remain unknown [84]. Over the coming years, we can anticipate that research efforts will be directed at answering important questions about selenoprotein function and the mechanisms by which these functions are linked to the health effects of dietary selenium and selenium metabolites in normal and disease states.

The idea that selenium-containing dietary supplements could be effective in preventing disease has gone through both optimistic and pessimistic phases. It has been recognized for decades that a deficiency of dietary selenium in certain populations is linked to cancer, cardiovascular disease, and various other disorders. Approximately 20 years ago, the Associated Press, Reuters, the New York Times, newspapers and media in many cities, and numerous Internet sites all enthusiastically reported the results of large epidemiological studies indicating that higher selenium levels are associated with a reduced risk of advanced prostate cancer. The subsequent failure of the very large and expensive SELECT trial a decade later greatly dampened the initial enthusiasm for the notion that simple dietary selenium supplementation could be an effective approach for cancer prevention. Some subsequent "postmortem" analyses of perceived flaws in the SELECT protocols did mitigate this disappointment to some extent, but it seems unlikely that government funding would be provided in the foreseeable future for any large-scale selenium supplementation trials similar to SELECT. As recently noted by Weekley and Harris [101], "with regards to the connection between selenium supplementation and disease prevention and treatment, there is an astounding gap between the

efficacy observed in laboratory studies and the mixed results of clinical trials. . .choice of selenium supplement speciation [*sic*] in laboratory and clinical studies may explain some of this efficacy gap." Another concern that also needs to be addressed in any future long-term supplementation protocol is the possibility of long-term dietary selenium toxicity. As well stated by Nogueira and Rocha [100], a balance must be struck in supplementation studies between nutritional requirements and the potential toxicological consequences of over- or under-selenium intake. Clearly, careful attention needs to be paid to the proper formulation and dosage of selenium dietary supplements.

Dietary supplementation on the one hand and the development of new therapeutic entities on the other are quite different matters. Glutathione peroxidase (GPx) was the first mammalian enzyme whose activity was clearly shown to be dependent on selenium. Since this selenoenzyme plays such an essential role in cellular defense against detrimental oxidants, a very strong impetus emerged for the development of selenium-containing small-molecule "GPx mimics" as potential therapeutic agents against disease states known to be associated with oxidative stress. In this regard, Roberts and coworkers [70] state as follows: "GPx activity has been previously observed to plateau with increased selenium administration, even as chemoprevention increases. . .this is one reason that it is no longer thought to play an important role in selenium-mediated chemoprevention." In this author's opinion, it is very unlikely that this statement holds true for all classes of selenium-based compounds. As detailed in this review, many current efforts continue to focus on compounds that modulate the glutathione-based – as well as the thioredoxin-based – antioxidant systems. Nevertheless, a general concern regarding the disappointing clinical outcomes to date of therapy with *natural* antioxidants in general must be kept in mind. As stated by Murphy [132], "a large number of well-conducted clinical trials have been carried out using several different antioxidants on a range of pathologies with little improvement in clinical outcome for the patients." Clearly, as Murphy himself concludes, more sophisticated drug design efforts are needed in the development of new chemical entities as antioxidants. It should be clear from the work described in this review that a number of such efforts with selenium-based compounds are underway.

In conclusion, it is quite evident that a number of selenium-based molecules with therapeutic potential are under development. While most of the efforts that entail *designed* organoselenium compounds – as opposed to inorganic selenium metabolites – are still at the preclinical stage, evidence is emerging that selenium-based compounds can operate by several biochemical and pharmacological mechanisms. Since our understanding of the biology, biochemistry, and pharmacology of selenium and selenoproteins is rapidly expanding, we can anticipate that the coming years will bring further development of new selenium-based pharmaceutical agents with therapeutic potential against human diseases.

References

1. Olcott HS, Brown WD, Van Derveen J (1961) Selenomethionine as an antioxidant. Nature 191:1201–1202
2. Walter R, Schwartz IL, Roy J (1972) Can selenoamino acids act as reversible biological antioxidants? Ann N Y Acad Sci 192:175–180
3. Beck MA, Shi Q, Morris VC, Levander OA (1995) Rapid genomic evolution of a non-virulent coxsackievirus B3 in selenium-deficient mice results in selection of identical virulent isolates. Nat Med 1(5):433–436
4. Levander OA, Beck MA (1997) Interacting nutritional and infectious etiologies of Keshan disease. Insights from coxsackie virus B-induced myocarditis in mice deficient in selenium or vitamin E. Biol Trace Elem Res 56(1):5–21. doi:10.1007/BF02778980
5. May SW (1999) Selenium-based drug design: rationale and therapeutic potential. Expert Opin Investig Drugs 8(7):1017–1030. doi:10.1517/13543784.8.7.1017
6. Shamberger RJ, Frost DV (1969) Possible protective effect of selenium against human cancer. Can Med Assoc J 100(14):682
7. Schrauzer GN, Rhead WJ (1971) Interpretation of the methylene blue reduction test of human plasma and the possible cancer protecting effect of selenium. Experientia 27(9):1069–1071
8. Clark LC, Cantor KP, Allaway WH (1991) Selenium in forage crops and cancer mortality in U.S. counties. Arch Environ Health 46(1):37–42. doi:10.1080/00039896.1991.9937427
9. Mugesh G, du Mont WW, Sies H (2001) Chemistry of biologically important synthetic organoselenium compounds. Chem Rev 101(7):2125–2179
10. Nogueira CW, Zeni G, Rocha JB (2004) Organoselenium and organotellurium compounds: toxicology and pharmacology. Chem Rev 104(12):6255–6285. doi:10.1021/cr0406559
11. May SW (2002) Selenium-based pharmacological agents: an update. Expert Opin Investig Drugs 11(9):1261–1269. doi:10.1517/13543784.11.9.1261
12. May SW, Pollock SH (1998) Selenium-based antihypertensives. Rationale and potential. Drugs 56(6):959–964
13. Kryukov GV, Castellano S, Novoselov SV, Lobanov AV, Zehtab O, Guigo R, Gladyshev VN (2003) Characterization of mammalian selenoproteomes. Science 300(5624):1439–1443. doi:10.1126/science.1083516
14. Yoshizawa K, Willett WC, Morris SJ, Stampfer MJ, Spiegelman D, Rimm EB, Giovannucci E (1998) Study of prediagnostic selenium level in toenails and the risk of advanced prostate cancer. J Natl Cancer Inst 90(16):1219–1224
15. van den Brandt PA, Zeegers MP, Bode P, Goldbohm RA (2003) Toenail selenium levels and the subsequent risk of prostate cancer: a prospective cohort study. Cancer Epidemiol Biomarkers Prev 12(9):866–871
16. Beck MA, Handy J, Levander OA (2004) Host nutritional status: the neglected virulence factor. Trends Microbiol 12(9):417–423. doi:10.1016/j.tim.2004.07.007
17. Beck MA, Levander OA, Handy J (2003) Selenium deficiency and viral infection. J Nutr 133 (5 Suppl 1):1463S–1467S
18. Duntas LH (2009) Selenium and inflammation: underlying anti-inflammatory mechanisms. Horm Metab Res 41(6):443–447. doi:10.1055/s-0029-1220724
19. Beckett GJ, Arthur JR (2005) Selenium and endocrine systems. J Endocrinol 184 (3):455–465. doi:10.1677/joe.1.05971
20. Council NR (2000) Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids. Washington, DC
21. Parnham M, Sies H (2000) Ebselen: prospective therapy for cerebral ischaemia. Expert Opin Investig Drugs 9(3):607–619. doi:10.1517/13543784.9.3.607
22. Clark LC, Combs GF Jr, Turnbull BW, Slate EH, Chalker DK, Chow J, Davis LS, Glover RA, Graham GF, Gross EG, Krongrad A, Lesher JL Jr, Park HK, Sanders BB Jr, Smith CL, Taylor JR (1996) Effects of selenium supplementation for cancer prevention in patients with

carcinoma of the skin. A randomized controlled trial .Nutritional Prevention of Cancer Study Group. JAMA 276(24):1957–1963
23. Lippman SM, Goodman PJ, Klein EA, Parnes HL, Thompson IM Jr, Kristal AR, Santella RM, Probstfield JL, Moinpour CM, Albanes D, Taylor PR, Minasian LM, Hoque A, Thomas SM, Crowley JJ, Gaziano JM, Stanford JL, Cook ED, Fleshner NE, Lieber MM, Walther PJ, Khuri FR, Karp DD, Schwartz GG, Ford LG, Coltman CA Jr (2005) Designing the selenium and vitamin E cancer prevention trial (SELECT). J Natl Cancer Inst 97(2):94–102. doi:10.1093/jnci/dji009
24. Ledesma MC, Jung-Hynes B, Schmit TL, Kumar R, Mukhtar H, Ahmad N (2011) Selenium and vitamin E for prostate cancer: post-SELECT (selenium and vitamin E cancer prevention trial) status. Mol Med 17(1–2):134–143. doi:10.2119/molmed.2010.00136
25. Lippman SM, Klein EA, Goodman PJ, Lucia MS, Thompson IM, Ford LG, Parnes HL, Minasian LM, Gaziano JM, Hartline JA, Parsons JK, Bearden JD 3rd, Crawford ED, Goodman GE, Claudio J, Winquist E, Cook ED, Karp DD, Walther P, Lieber MM, Kristal AR, Darke AK, Arnold KB, Ganz PA, Santella RM, Albanes D, Taylor PR, Probstfield JL, Jagpal TJ, Crowley JJ, Meyskens FL Jr, Baker LH, Coltman CA Jr (2009) Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the selenium and vitamin E cancer prevention trial (SELECT). JAMA 301(1):39–51. doi:10.1001/jama.2008.864
26. May SW, Herman HH, Roberts SF, Ciccarello MC (1987) Ascorbate depletion as a consequence of product recycling during dopamine beta-monooxygenase catalyzed selenoxidation. Biochemistry 26(6):1626–1633
27. May SW, Wimalasena K, Herman HH, Fowler LC, Ciccarello MC, Pollock SH (1988) Novel antihypertensives targeted at dopamine beta-monooxygenase: turnover-dependent cofactor depletion by phenyl aminoethyl selenide. J Med Chem 31(6):1066–1068
28. Herman HH, Wimalasena K, Fowler LC, Beard CA, May SW (1988) Demonstration of the ascorbate dependence of membrane-bound dopamine beta-monooxygenase in adrenal chromaffin granule ghosts. J Biol Chem 263(2):666–672
29. Wimalasena K, Herman HH, May SW (1989) Effects of dopamine beta-monooxygenase substrate analogs on ascorbate levels and norepinephrine synthesis in adrenal chromaffin granule ghosts. J Biol Chem 264(1):124–130
30. Pollock SH, Herman HH, Fowler LC, Edwards AS, Evans CO, May SW (1988) Demonstration of the antihypertensive activity of phenyl-2-aminoethyl selenide. J Pharmacol Exp Ther 246(1):227–234
31. Herman HH, Pollock SH, Fowler LC, May SW (1988) Demonstration of the potent antihypertensive activity of phenyl-2-aminoethyl sulfides. J Cardiovasc Pharmacol 11(5):501–510
32. May SW, Wang L, Gill-Woznichak MM, Browner RF, Ogonowski AA, Smith JB, Pollock SH (1997) An orally active selenium-based antihypertensive agent with restricted CNS permeability. J Pharmacol Exp Ther 283(2):470–477
33. Overcast JD, Ensley AE, Buccafusco CJ, Cundy C, Broadnax RA, He S, Yoganathan AP, Pollock SH, Hartley CJ, May SW (2001) Evaluation of cardiovascular parameters of a selenium-based antihypertensive using pulsed Doppler ultrasound. J Cardiovasc Pharmacol 38(3):337–346
34. De Silva V, Woznichak MM, Burns KL, Grant KB, May SW (2004) Selenium redox cycling in the protective effects of organoselenides against oxidant-induced DNA damage. J Am Chem Soc 126(8):2409–2413. doi:10.1021/ja037294j
35. Woznichak MM, Overcast JD, Robertson K, Neumann HM, May SW (2000) Reaction of phenylaminoethyl selenides with peroxynitrite and hydrogen peroxide. Arch Biochem Biophys 379(2):314–320. doi:10.1006/abbi.2000.1893
36. Cowan EA, Oldham CD, May SW (2011) Identification of a thioselenurane intermediate in the reaction between phenylaminoalkyl selenoxides and glutathione. Arch Biochem Biophys 506(2):201–207. doi:10.1016/j.abb.2010.11.007
37. Cowan EA, Taylor JL, Oldham CD, Dasari M, Doyle D, Murthy N, May SW (2013) Cellular antioxidant activity of phenylaminoethyl selenides as monitored by chemiluminescence of

peroxalate nanoparticles and by reduction of lipopolysaccharide-induced oxidative stress. Enzyme Microb Technol 53(6–7):373–377. doi:10.1016/j.enzmictec.2013.08.002
38. Kumar S, Singh HB, Wolmershauser G (2006) Protection against peroxynitrite-mediated nitration reaction by intramolecularly coordinated diorganoselenides. Organometallics 25:382–393
39. Reich HJ, Jasperse CP (1987) Organoselenium chemistry. Redox chemistry of selenocysteine model systems. J Am Chem Soc 109(18):5549–5551
40. Back TG, Moussa Z, Parvez M (2004) The exceptional glutathione peroxidase-like activity of di(3-hydroxypropyl) selenide and the unexpected role of a novel spirodioxaselenanonane intermediate in the catalytic cycle. Angew Chem Int Ed 43:1268–1270
41. Thannickal VJ, Fanburg BL (2000) Reactive oxygen species in cell signaling. Am J Physiol Lung Cell Mol Physiol 279(6):L1005–L1028
42. Sarsour EH, Kumar MG, Chaudhuri L, Kalen AL, Goswami PC (2009) Redox control of the cell cycle in health and disease. Antioxid Redox Signal 11(12):2985–3011. doi:10.1089/ARS.2009.2513
43. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB (2010) Oxidative stress, inflammation, and cancer: how are they linked? Free Radic Biol Med 49(11):1603–1616. doi:10.1016/j.freeradbiomed.2010.09.006
44. Hande KR (1998) Clinical applications of anticancer drugs targeted to topoisomerase II. Biochim Biophys Acta 1400(1–3):173–184
45. Quiles JL, Huertas JR, Battino M, Mataix J, Ramirez-Tortosa MC (2002) Antioxidant nutrients and adriamycin toxicity. Toxicology 180(1):79–95
46. Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L (2004) Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacol Rev 56(2):185–229. doi:10.1124/pr.56.2.6
47. Pearlman M, Jendiroba D, Pagliaro L, Keyhani A, Liu B, Freireich EJ (2003) Dexrazoxane in combination with anthracyclines lead to a synergistic cytotoxic response in acute myelogenous leukemia cell lines. Leuk Res 27(7):617–626
48. Tebbi CK, London WB, Friedman D, Villaluna D, De Alarcon PA, Constine LS, Mendenhall NP, Sposto R, Chauvenet A, Schwartz CL (2007) Dexrazoxane-associated risk for acute myeloid leukemia/myelodysplastic syndrome and other secondary malignancies in pediatric Hodgkin's disease. J Clin Oncol 25(5):493–500. doi:10.1200/JCO.2005.02.3879
49. Kang JY, Costyn LJ, Nagy T, Cowan EA, Oldham CD, May SW, Arnold RD (2011) The antioxidant phenylaminoethyl selenide reduces doxorubicin-induced cardiotoxicity in a xenograft model of human prostate cancer. Arch Biochem Biophys 515(1–2):112–119. doi:10.1016/j.abb.2011.08.008
50. Parnham M (1996) The pharmaceutical potential of selenium. Pharmaceut News 3:7–10
51. Parnham MJ, Sies H (2013) The early research and development of ebselen. Biochem Pharmacol 86(9):1248–1253. doi:10.1016/j.bcp.2013.08.028
52. Back TG, Moussa Z, Parvez M (2004) The exceptional glutathione peroxidase-like activity of di(3-hydroxypropyl) selenide and the unexpected role of a novel spirodioxaselenanonane intermediate in the catalytic cycle. Angew Chem Int Ed 43(10):1268–1270. doi:10.1002/anie.200353128
53. Kunwar A, Mishra B, Barik A, Kumbhare LB, Pandey R, Jain VK, Priyadarsini KI (2007) 3,3′-diselenodipropionic acid, an efficient peroxyl radical scavenger and a GPx mimic, protects erythrocytes (RBCs) from AAPH-induced hemolysis. Chem Res Toxicol 20 (10):1482–1487. doi:10.1021/tx700137a
54. Sarma BK, Mugesh G (2008) Antioxidant activity of the anti-inflammatory compound ebselen: a reversible cyclization pathway via selenenic and seleninic acid intermediates. Chemistry 14(34):10603–10614. doi:10.1002/chem.200801258
55. Back TG, Moussa Z (2003) Diselenides and allyl selenides as glutathione peroxidase mimetics. Remarkable activity of cyclic seleninates produced in situ by the oxidation of

allyl omega-hydroxyalkyl selenides. J Am Chem Soc 125(44):13455–13460. doi:10.1021/ja0357588
56. Sarma BK, Mugesh G (2005) Glutathione peroxidase (GPx)-like antioxidant activity of the organoselenium drug ebselen: unexpected complications with thiol exchange reactions. J Am Chem Soc 127(32):11477–11485. doi:10.1021/ja052794t
57. Davies R, Bartholomeusz DA, Andrade J (2003) Personal sensors for the diagnosis and management of metabolic disorders. IEEE Eng Med Biol Mag 22(1):32–42
58. Balkrishna SJ, Kumar S, Azad GK, Bhakuni BS, Panini P, Ahalawat N, Tomar RS, Detty MR, Kumar S (2014) An ebselen like catalyst with enhanced GPx activity via a selenol intermediate. Org Biomol Chem 12(8):1215–1219. doi:10.1039/c4ob00027g
59. Yamaguchi T, Sano K, Takakura K, Saito I, Shinohara Y, Asano T, Yasuhara H (1998) Ebselen in acute ischemic stroke: a placebo-controlled, double-blind clinical trial. Ebselen Study Group. Stroke 29(1):12–17
60. Kono H, Arteel GE, Rusyn I, Sies H, Thurman RG (2001) Ebselen prevents early alcohol-induced liver injury in rats. Free Radic Biol Med 30(4):403–411
61. Moussaoui S, Obinu MC, Daniel N, Reibaud M, Blanchard V, Imperato A (2000) The antioxidant ebselen prevents neurotoxicity and clinical symptoms in a primate model of Parkinson's disease. Exp Neurol 166(2):235–245. doi:10.1006/exnr.2000.7516
62. Chew P, Yuen DY, Stefanovic N, Pete J, Coughlan MT, Jandeleit-Dahm KA, Thomas MC, Rosenfeldt F, Cooper ME, de Haan JB (2010) Antiatherosclerotic and renoprotective effects of ebselen in the diabetic apolipoprotein E/GPx1-double knockout mouse. Diabetes 59 (12):3198–3207. doi:10.2337/db10-0195
63. Chander PN, Gealekman O, Brodsky SV, Elitok S, Tojo A, Crabtree M, Gross SS, Goligorsky MS (2004) Nephropathy in Zucker diabetic fat rat is associated with oxidative and nitrosative stress: prevention by chronic therapy with a peroxynitrite scavenger ebselen. J Am Soc Nephrol 15(9):2391–2403. doi:10.1097/01.ASN.0000135971.88164.2C
64. He M, Xing S, Yang B, Zhao L, Hua H, Liang Z, Zhou W, Zeng J, Pei Z (2007) Ebselen attenuates oxidative DNA damage and enhances its repair activity in the thalamus after focal cortical infarction in hypertensive rats. Brain Res 1181:83–92. doi:10.1016/j.brainres.2007.08.072
65. Yamagata K, Ichinose S, Miyashita A, Tagami M (2008) Protective effects of ebselen, a seleno-organic antioxidant on neurodegeneration induced by hypoxia and reperfusion in stroke-prone spontaneously hypertensive rat. Neuroscience 153(2):428–435. doi:10.1016/j.neuroscience.2008.02.028
66. Moutet M, d'Alessio P, Malette P, Devaux V, Chaudiere J (1998) Glutathione peroxidase mimics prevent TNFalpha- and neutrophil-induced endothelial alterations. Free Radic Biol Med 25(3):270–281
67. Erdelmeier I, Chaudiere J, Moutet M, Yadan J-C (1999) Compounds having a benzisoselenazoline and -azine structure, method for preparing same and therapeutic uses thereof. United States Patent 5,968,920
68. Lange RW (2000) BXT-51072 OXIS international. Curr Opin Investig Drugs 2(4):338–341
69. Oxis International, Inc. (2005) Form 10-QSB quarterly report pursuant to Section 13 or 15 (d) of the Securities Exchange Act of 1934 for the quarterly period ended March 31, 2005
70. Short MD, Xie Y, Li L, Cassidy PB, Roberts JC (2003) Characteristics of selenazolidine prodrugs of selenocysteine: toxicity and glutathione peroxidase induction in V79 cells. J Med Chem 46(15):3308–3313. doi:10.1021/jm020496q
71. Li L, Xie Y, El-Sayed WM, Szakacs JG, Roberts JC (2004) Characteristics of selenazolidine prodrugs of selenocysteine: toxicity, selenium levels, and glutathione peroxidase induction in A/J mice. Life Sci 75(4):447–459. doi:10.1016/j.lfs.2003.12.018
72. Franklin MR, Moos PJ, El-Sayed WM, Aboul-Fadl T, Roberts JC (2007) Pre- and post-initiation chemoprevention activity of 2-alkyl/aryl selenazolidine-4(R)-carboxylic acids against tobacco-derived nitrosamine (NNK)-induced lung tumors in the A/J mouse. Chem Biol Interact 168(3):211–220. doi:10.1016/j.cbi.2007.04.012

73. Poerschke RL, Moos PJ (2011) Thioredoxin reductase 1 knockdown enhances selenazolidine cytotoxicity in human lung cancer cells via mitochondrial dysfunction. Biochem Pharmacol 81(2):211–221. doi:10.1016/j.bcp.2010.09.024
74. Spallholz JE, Reid TW (1998) Method for the preparation of free radical pharmaceuticals using selenium conjugates. United States Patent 5,783,454
75. Spallholz JE, Reid TW (2000) Method for the preparation of free radical pharmaceuticals diagnostics and devices using selenium conjugates United States Patent 6,077,714
76. Spallholz JE, Reid TW (1999) Selenium carrier conjugates. United States Patent 5,994,151
77. Tran PL, Hammond AA, Mosley T, Cortez J, Gray T, Colmer-Hamood JA, Shashtri M, Spallholz JE, Hamood AN, Reid TW (2009) Organoselenium coating on cellulose inhibits the formation of biofilms by *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Appl Environ Microbiol 75(11):3586–3592. doi:10.1128/AEM. 02683-08
78. Tran P, Hamood A, Mosley T, Gray T, Jarvis C, Webster D, Amaechi B, Enos T, Reid T (2013) Organo-selenium-containing dental sealant inhibits bacterial biofilm. J Dent Res 92 (5):461–466. doi:10.1177/0022034513482141
79. Kunstelj M, Menart V, Ambrozic G, Gaberc-Porekar V (2013) Selenium containing modifying agents and conjugates. United States Patent 8,389,695
80. Miki K, Xu M, Tan Y (2005) Selenium-containing pro-drugs for cancer therapy. United States Patent 6,916,793
81. Powis G, Oblong JE, Gasdaska PY, Berggren M, Hill SR, Kirkpatrick DL (1994) The thioredoxin/thioredoxin reductase redox system and control of cell growth. Oncol Res 6 (10–11):539–544
82. Biguet C, Wakasugi N, Mishal Z, Holmgren A, Chouaib S, Tursz T, Wakasugi H (1994) Thioredoxin increases the proliferation of human B-cell lines through a protein kinase C-dependent mechanism. J Biol Chem 269(46):28865–28870
83. Fernandes AP, Capitanio A, Selenius M, Brodin O, Rundlof AK, Bjornstedt M (2009) Expression profiles of thioredoxin family proteins in human lung cancer tissue: correlation with proliferation and differentiation. Histopathology 55(3):313–320. doi:10.1111/j.1365-2559.2009.03381.x
84. Hatfield DL, Tsuji PA, Carlson BA, Gladyshev VN (2014) Selenium and selenocysteine: roles in cancer, health, and development. Trends Biochem Sci 39(3):112–120. doi:10.1016/j.tibs.2013.12.007
85. Yoo MH, Xu XM, Carlson BA, Gladyshev VN, Hatfield DL (2006) Thioredoxin reductase 1 deficiency reverses tumor phenotype and tumorigenicity of lung carcinoma cells. J Biol Chem 281(19):13005–13008. doi:10.1074/jbc.C600012200
86. Engman L, Cotgreave I, Angulo M, Taylor CW, Paine-Murrieta GD, Powis G (1997) Diaryl chalcogenides as selective inhibitors of thioredoxin reductase and potential antitumor agents. Anticancer Res 17(6D):4599–4605
87. Engman L, Kandra T, Gallegos A, Williams R, Powis G (2000) Water-soluble organotellurium compounds inhibit thioredoxin reductase and the growth of human cancer cells. Anticancer Drug Des 15(5):323–330
88. Zhao R, Masayasu H, Holmgren A (2002) Ebselen: a substrate for human thioredoxin reductase strongly stimulating its hydroperoxide reductase activity and a superfast thioredoxin oxidant. Proc Natl Acad Sci U S A 99(13):8579–8584. doi:10.1073/pnas.122061399
89. Lu J, Holmgren A (2014) The thioredoxin antioxidant system. Free Radic Biol Med 66:75–87. doi:10.1016/j.freeradbiomed.2013.07.036
90. Casagrande S, Bonetto V, Fratelli M, Gianazza E, Eberini I, Massignan T, Salmona M, Chang G, Holmgren A, Ghezzi P (2002) Glutathionylation of human thioredoxin: a possible crosstalk between the glutathione and thioredoxin systems. Proc Natl Acad Sci U S A 99 (15):9745–9749. doi:10.1073/pnas.152168599
91. Wang L, Yang Z, Fu J, Yin H, Xiong K, Tan Q, Jin H, Li J, Wang T, Tang W, Yin J, Cai G, Liu M, Kehr S, Becker K, Zeng H (2012) Ethaselen: a potent mammalian thioredoxin

reductase 1 inhibitor and novel organoselenium anticancer agent. Free Radic Biol Med 52 (5):898–908. doi:10.1016/j.freeradbiomed.2011.11.034
92. Li DD, He J, Zeng HH (2012) Biological evaluation of novel selenazole-based compounds as potential thioredoxin reductase inhibitors. Appl Organomet Chem 26:619–624. doi:10.1002/aoc.2910
93. Baker AF, Adab KN, Raghunand N, Chow HH, Stratton SP, Squire SW, Boice M, Pestano LA, Kirkpatrick DL, Dragovich T (2013) A phase IB trial of 24-hour intravenous PX-12, a thioredoxin-1 inhibitor, in patients with advanced gastrointestinal cancers. Invest New Drugs 31(3):631–641. doi:10.1007/s10637-012-9846-2
94. Traynor AM, Thomas JP, Ramanathan RK, Mody TD, Alberti D, Wilding G, Bailey HH (2011) Phase I trial of motexafin gadolinium and doxorubicin in the treatment of advanced malignancies. Invest New Drugs 29(2):316–322. doi:10.1007/s10637-009-9364-z
95. Edelman MJ, Otterson G, Leach J, Malpass T, Salgia R, Jones D, Mody TD, Govindan R (2011) Multicenter phase II trial of Motexafin gadolinium and pemetrexed for second-line treatment in patients with non-small cell lung cancer. J Thorac Oncol 6(4):786–789. doi:10.1097/JTO.0b013e31820a443f
96. Mahmood DF, Abderrazak A, El Hadri K, Simmet T, Rouis M (2013) The thioredoxin system as a therapeutic target in human health and disease. Antioxid Redox Signal 19 (11):1266–1303. doi:10.1089/ars.2012.4757
97. Combs GF Jr, Gray WP (1998) Chemopreventive agents: selenium. Pharmacol Ther 79 (3):179–192
98. Combs GF Jr (1999) Chemopreventive mechanisms of selenium. Med Klin 94(Suppl 3):18–24
99. Ganther HE (1999) Selenium metabolism, selenoproteins and mechanisms of cancer prevention: complexities with thioredoxin reductase. Carcinogenesis 20(9):1657–1666
100. Nogueira CW, Rocha JB (2011) Toxicology and pharmacology of selenium: emphasis on synthetic organoselenium compounds. Arch Toxicol 85(11):1313–1359. doi:10.1007/s00204-011-0720-3
101. Weekley CM, Harris HH (2013) Which form is that? The importance of selenium speciation and metabolism in the prevention and treatment of disease. Chem Soc Rev 42 (23):8870–8894. doi:10.1039/c3cs60272a
102. Lanfear J, Fleming J, Wu L, Webster G, Harrison PR (1994) The selenium metabolite selenodiglutathione induces p53 and apoptosis: relevance to the chemopreventive effects of selenium? Carcinogenesis 15(7):1387–1392
103. Cho DY, Jung U, Chung AS (1999) Induction of apoptosis by selenite and selenodiglutathione in HL-60 cells: correlation with cytotoxicity. Biochem Mol Biol Int 47(5):781–793
104. Ip C, Ganther HE (1992) Comparison of selenium and sulfur analogs in cancer prevention. Carcinogenesis 13(7):1167–1170
105. Spyrou G, Bjornstedt M, Kumar S, Holmgren A (1995) AP-1 DNA-binding activity is inhibited by selenite and selenodiglutathione. FEBS Lett 368(1):59–63
106. Ren X, Bjornstedt M, Shen B, Ericson ML, Holmgren A (1993) Mutagenesis of structural half-cystine residues in human thioredoxin and effects on the regulation of activity by selenodiglutathione. Biochemistry 32(37):9701–9708
107. Bjornstedt M, Kumar S, Holmgren A (1995) Selenite and selenodiglutathione: reactions with thioredoxin systems. Methods Enzymol 252:209–219
108. Nilsonne G, Olm E, Szulkin A, Mundt F, Stein A, Kocic B, Rundlof AK, Fernandes AP, Bjornstedt M, Dobra K (2009) Phenotype-dependent apoptosis signalling in mesothelioma cells after selenite exposure. J Exp Clin Cancer Res 28:92. doi:10.1186/1756-9966-28-92
109. Asfour IA, El-Tehewi MM, Ahmed MH, Abdel-Sattar MA, Moustafa NN, Hegab HM, Fathey OM (2009) High-dose sodium selenite can induce apoptosis of lymphoma cells in adult patients with non-Hodgkin's lymphoma. Biol Trace Elem Res 127(3):200–210. doi:10.1007/s12011-008-8240-6

110. Wang L, Bonorden MJ, Li GX, Lee HJ, Hu H, Zhang Y, Liao JD, Cleary MP, Lu J (2009) Methyl-selenium compounds inhibit prostate carcinogenesis in the transgenic adenocarcinoma of mouse prostate model with survival benefit. Cancer Prev Res 2(5):484–495. doi:10.1158/1940-6207.CAPR-08-0173
111. Yan L, DeMars LC (2012) Dietary supplementation with methylseleninic acid, but not selenomethionine, reduces spontaneous metastasis of Lewis lung carcinoma in mice. Int J Cancer 131(6):1260–1266. doi:10.1002/ijc.27355
112. Hu H, Jiang C, Ip C, Rustum YM, Lu J (2005) Methylseleninic acid potentiates apoptosis induced by chemotherapeutic drugs in androgen-independent prostate cancer cells. Clin Cancer Res 11(6):2379–2388. doi:10.1158/1078-0432.CCR-04-2084
113. Li Z, Carrier L, Belame A, Thiyagarajah A, Salvo VA, Burow ME, Rowan BG (2009) Combination of methylselenocysteine with tamoxifen inhibits MCF-7 breast cancer xenografts in nude mice through elevated apoptosis and reduced angiogenesis. Breast Cancer Res Treat 118(1):33–43. doi:10.1007/s10549-008-0216-x
114. Ip C, Thompson HJ, Zhu Z, Ganther HE (2000) In vitro and in vivo studies of methylseleninic acid: evidence that a monomethylated selenium metabolite is critical for cancer chemoprevention. Cancer Res 60(11):2882–2886
115. Bhattacharya A (2011) Methylselenocysteine: a promising antiangiogenic agent for overcoming drug delivery barriers in solid malignancies for therapeutic synergy with anticancer drugs. Expert Opin Drug Deliv 8(6):749–763. doi:10.1517/17425247.2011.571672
116. Hoffman JL, McConnell KP, Carpenter DR (1970) Aminoacylation of Escherichia coli methionine tRNA by selenomethionine. Biochim Biophys Acta 199(2):531–534
117. Redman C, Scott JA, Baines AT, Basye JL, Clark LC, Calley C, Roe D, Payne CM, Nelson MA (1998) Inhibitory effect of selenomethionine on the growth of three selected human tumor cell lines. Cancer Lett 125(1–2):103–110
118. Burke KE, Combs GF Jr, Gross EG, Bhuyan KC, Abu-Libdeh H (1992) The effects of topical and oral L-selenomethionine on pigmentation and skin cancer induced by ultraviolet irradiation. Nutr Cancer 17(2):123–137. doi:10.1080/01635589209514180
119. Frenkel GD, Caffrey PB (2001) A prevention strategy for circumventing drug resistance in cancer chemotherapy. Curr Pharm Des 7(16):1595–1614
120. Caffrey PB, Frenkel GD (2000) Selenium compounds prevent the induction of drug resistance by cisplatin in human ovarian tumor xenografts in vivo. Cancer Chemother Pharmacol 46(1):74–78. doi:10.1007/s002800000127
121. Miki K, Xu M, Gupta A, Ba Y, Tan Y, Al-Refaie W, Bouvet M, Makuuchi M, Moossa AR, Hoffman RM (2001) Methioninase cancer gene therapy with selenomethionine as suicide prodrug substrate. Cancer Res 61(18):6805–6810
122. Pinton S, Bruning CA, Sartori Oliveira CE, Prigol M, Nogueira CW (2013) Therapeutic effect of organoselenium dietary supplementation in a sporadic dementia of Alzheimer's type model in rats. J Nutr Biochem 24(1):311–317. doi:10.1016/j.jnutbio.2012.06.012
123. Pinton S, Souza AC, Sari MH, Ramalho RM, Rodrigues CM, Nogueira CW (2013) p, p′-Methoxyl-diphenyl diselenide protects against amyloid-beta induced cytotoxicity in vitro and improves memory deficits in vivo. Behav Brain Res 247:241–247. doi:10.1016/j.bbr.2013.03.034
124. Shin KM, Shen L, Park SJ, Jeong JH, Lee KT (2009) Bis-(3-hydroxyphenyl) diselenide inhibits LPS-stimulated iNOS and COX-2 expression in RAW 264.7 macrophage cells through the NF-kappaB inactivation. J Pharm Pharmacol 61(4):479–486. doi:10.1211/jpp/61.04.0010
125. Zasso FB, Goncales CE, Jung EA, Araldi D, Zeni G, Rocha JB, Nogueira CW (2005) On the mechanisms involved in antinociception induced by diphenyl diselenide. Environ Toxicol Pharmacol 19(2):283–289. doi:10.1016/j.etap.2004.08.003
126. Nogueira CW, Rocha JBT (2010) Diphenyl diselenide a Janus-faced molecule. J Braz Chem Soc 21(11):2055–2071. doi:10.1016/j.bbr.2013.03.034

127. Iwaoka M, Kenta A (2013) From sulfur to selenium. A new research arena in chemical biology and biological chemistry. Curr Chem Biol 7:2–14
128. Reich HJ (1978) Organoselenium oxidations. Oxidation in organic chemistry part C, pp 1–130
129. Xiao H, Parkin KL (2006) Induction of phase II enzyme activity by various selenium compounds. Nutr Cancer 55(2):210–223. doi:10.1207/s15327914nc5502_13
130. Steinmann D, Nauser T, Beld J, Tanner M, Gunther D, Bounds PL, Koppenol WH (2008) Kinetics of tyrosyl radical reduction by selenocysteine. Biochemistry 47(36):9602–9607. doi:10.1021/bi801029f
131. Labunskyy VM, Hatfield DL, Gladyshev VN (2014) Selenoproteins: molecular pathways and physiological roles. Physiol Rev 94(3):739–777. doi:10.1152/physrev.00039.2013
132. Murphy MP (2014) Antioxidants as therapies: can we improve on nature? Free Radic Biol Med 66:20–23. doi:10.1016/j.freeradbiomed.2013.04.010