Selenium Compounds and Apoptotic Modulation: A New Perspective in Cancer Therapy

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Abstract: Recent epidemiological studies have demonstrated that selenium may be an effective chemopreventive and anticancer agent with a broad spectrum against several human cancer cells (prostate, colon, bladder, lung, liver, ovarian, leukemia). A wide range of potential mechanisms have been proposed for the antitumorigenic effects of selenium and these include antiandrogen activity, growth inhibitory effects by regulation of p53 and antioxidant function, and through DNA damage. However, apoptosis is one of the most plausible mechanisms for the anticancer activity. The regulating mechanisms of apoptosis are extremely complex and for selenium compounds they mainly involve a mitochondrial pathway, protein kinases, tumor necrosis factor, activation of caspases and reactive oxygen species. The aim of this review is to summarize the current knowledge about more than twenty eight selenium-containing molecules and to discuss the implications for apoptosis and the impact in cancer therapy.

Key Words: Selenium, cancer, cytotoxicity, apoptosis.

1. INTRODUCTION

Worldwide, there are more than 10 million new cancer cases each year, and cancer is the cause of approximately 12% of all deaths. Given these facts, a large number of epidemiologic studies have been undertaken to identify potential risk factors for cancer, amongst which the association with trace elements [1-5] such as selenium has received considerable attention. The anticarcinogenic potential of selenium was first identified nearly 40 years ago in geographical studies that highlighted lower death rates due to cancer in regions with high levels of selenium. In recent years this appreciation has grown and considerable efforts have been made to identify new compounds, mechanisms of action and perspectives of this approach. Selenium is found naturally in the environment and human exposure derives from a variety of sources, including air, drinking water and food [6,7]. Trace elements are of particular interest given that the levels of exposure to them are potentially modifiable [8]. The recommended dietary Se amount in humans is 35-70 μg/day (National Institutes of Health, NIH). Overall, the evidence currently available appears to support an inverse association between selenium exposure and prostate cancer risk [9,10], bladder [11] and possibly also a reduction in risk with respect to lung cancer [12], although additional prospective studies are needed. However, the high recurrence rate and ability to monitor bladder [13] urothelial-cell carcinoma make selenium a good candidate for chemoprevention. In relation to colorectal cancer [14], which is the third most frequent fatal malignant neoplasm in the United States, intervention strategies with selenium compounds represent a viable option to reduce colon cancer. In addition, selenium deficiency is associated with different diseases, including liver necrosis [15], and is protective against viral hepatitis and hepatocellular carcinoma (HCC). Furthermore, some selenium compounds were shown to have the ability to inhibit gene expression [16] correlated with the incidence of large tumors. Recently, Rooprai *et al.* [17] reported that selenium induces the inhibition of invasion in brain tumor cells. In addition, Zuo *et al.* [18] discovered that selenium was involved in protection against leukemia by control oxidative stress.

This review includes information on twenty eight general chemical structures containing selenium that have been reported to have either anticancer, chemopreventive or apoptotic activities. Thus, selenium compounds emerge as promising downstream candidates for cancer therapy.

2. SELENIUM COMPOUNDS AND ANTICANCER ACTIVITY

The anticancer activity of selenium is dependent on its chemical form. In general, inorganic selenium compounds, such as selenate or selenite, are known to produce genotoxic effects and are therefore not preferred for medicinal use – especially at high doses. The results indicate that the genotoxic and antigenotoxic properties of selenium compounds are highly dependent upon the conditions under which they are evaluated [19]. Organic selenium-containing compounds are better tolerated, but differ in their anticancer activity depending on dosage, their pharmacokinetic and pharmacodynamic properties. So, diphenyl diselenide [20] (DPDS) presents toxic effects as it inhibits δ-aminolevulinate dehydratase in several tissues and organs. Moreover, chronic exposure of the mouse brain to high doses of DPDS affects the central nervous system, impairment of glutamatergic transmission, and excitotoxicity, as well as liver and renal toxicity. In microorganism models, DPDS was able to induce frameshift mutations in both *Salmonella typhimurium* and haploid yeast, as well as to increase crossing over and gene conversion frequencies in diploid strains of *Saccharomyces cerevisiae*. At low doses this molecule has antioxidant prop-

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erties, protecting the cells against oxidative damage. Both inorganic and organic forms of selenium can be utilized as nutritional and supplemental selenium sources, and selenite and selenate are typical inorganic ones. On the other hand, organic selenium is present in general as selenoamino acids such as, selenocysteine (SeCys), selenomethionine (SeMet) and methylselenocysteine (MeSeCys). The metabolism of selenium (Fig. **1**) [21] to a monomethylated intermediate, methylselenol, is presumed to be necessary for the expression of anticancer activity. Methylselenol (CH_3SeH) is highly reactive and difficult to formulate. Stable precursors, such as methylselenocysteine (MeSeCys), methylseleninic acid (MSA) or selenomethionine (SeMet), which can be converted endogenously to methylselenol, are good selenium precursors for the generation of this metabolite that exhibits anticarcinogenic activity.

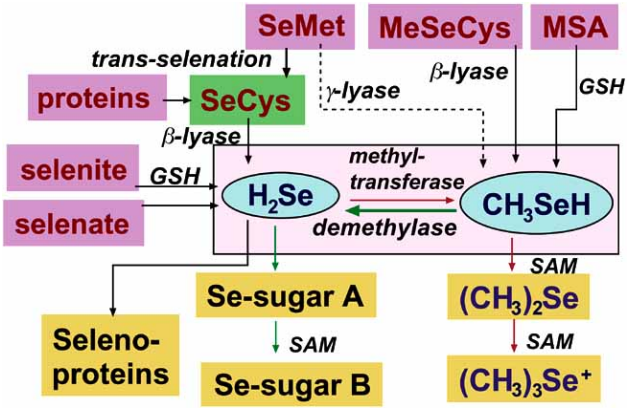


Fig. (1).

Se-Methylated selenoamino acids SeMet and methylselenocysteine (MeSeCys) are chemically less reactive forms compared with the corresponding non-Se-methylated selenoamino acids. In addition to these typical natural nutritional selenium sources, methylseleninic acids (MSA) has been shown to be incorporated into selenoproteins and excretion metabolites selenosugar and trimethylselenonium (TMSe), As all inorganic and organic nutritional selenium sources have been utilized for the syntheses of selenoproteins for biological use and selenosugar for excretion, they have been deduced to be transformed into the common intermediate selenide, as schematically shown in (Fig.**1**). On the other hand, selenoamino acids are transformed into selenide through the lyase reaction; SeCys liberated from selenoproteins is transformed into selenide through the β-lyase reaction (SeCys→H_2Se), whereas SeMet is transformed into selenide either through the trans-selenation pathway into SeCys followed by the β-lyase reaction (SeMet→SeCys→H_2Se) or directly through the γ-lyase reaction into methylselenol followed by the demethylation reaction (SeMet→CH_3SeH→H_2Se). A monomeric form of Se-methylated selenoamino acid, MeSeCys, is assumed to be transformed into selenide through the β-lyase reaction to produce methylselenol, and then into selenide through demethylation reaction (MeSeCys→CH_3SeH→H_2Se). In addition, MSA has been proposed to be reduced to methylselenol, and then transformed through the demethylation reaction into selenide $CH_3Se(O)OH$→CH_3SeH→H_2Se). The equilibrium between selenide and methylselenol is dependent on the methylation and demethylation activities, and also on the removal of selenium in the forms of selenoproteins and selenosugar.

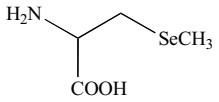
2.1. Antiandrogen Activity

Antiandrogenic drugs, or more appropriately androgen-receptor antagonists, represent a group of compounds that have played a limited role in the treatment of metastatic prostate cancer. The mode of action of these compounds is primarily one that involves blocking androgens at their receptor sites in target tissues. Antiandrogenics have been used in numerous trials both in Europe and the United States. This group of compounds has been used in monotherapy and in combination therapy. Sodium selenite and methylseleninic acid (MSA) (Fig. **2**) are the two most studied selenium compounds for prostate cancer. Both of these compounds may modulate the androgen receptor (AR). Among the mechanisms of action implicated for sodium selenite is the repression of interleukin-6 (IL-6) [22]. It was found that when human prostate cancer LNCaP cells were exposed to sodium selenite in the presence of IL-6, a reduction in cell growth was observed in conjunction with a significant inhibition of prostate specific antigen (PSA) protein expression. Methylseleninic acid [23] causes disruption of AR signalling by reaction with reduced glutathione within the prostate cancer cell. In addition, it was observed that a combination of methylseleninic acid with alpha-tocopheryl succinate [24] produced a greater than additive effect in suppressing AR signalling compared with the single agent.

Methylseleninic acid Sodium selenite

Fig. (2).

Methylselenocysteine (MeSeCys) (Fig. **3**) significantly inhibited LNCaP tumor growth and also reduced AR expression in tumor tissues [25] and serum PSA levels in mice treated with this compound.

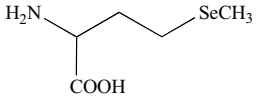


Methylselenocysteine

Fig. (3).

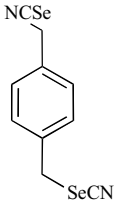
Pinto [26] later reported a study aimed at comparing the effects of naturally occurring methylselenocysteine, selenomethionine (SeMet) (Fig. **4**) and synthetic 1,4-phenylenebis (methylene)selenocyanate (*p*-XSC) (Fig. **5**) and p-xylylbis (methylselenide) (*p*-XMS) (Fig. **6**) organoselenium compounds in androgen responsive LNCaP and its androgen-independent clone LNCaP C4-2. Depending on the structure, these molecules exhibit differential effects on growth and PSA secretion. For example, SeMet and MeSeCys led to a significant decrease in growth at high doses whereas *p*-XSC and *p*-XMS gave an effect at low doses. The largest de-

creases in PSA levels were obtained with *p*-XMS, whereas MeSeCys had no effect.



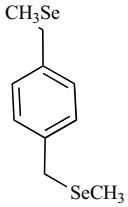
Selenomethionine

Fig. (4).



p-XSC

Fig. (5).



p-XMS

Fig. (6).

2.2. Antioxidant Function

Although the chemopreventive activity of selenium compounds has been attributed to a variety of molecular targets, a link between the antitumorigenic action and positive effects on protective enzymes remains a recurrent theme. Some protective enzymes, such as gluthatione peroxidase (GPx) and thioredoxin reductase, are selenoproteins and are likely to be impacted by selenium supplementation, but there are others, like NAD(P)*H*-quinone oxidoreductase and glutathione transferase, where any requirement for selenium is less obvious. Glutathione peroxidase (GPx) [27] catalyzes the reduction of H_2O_2 and harmful organic peroxides by glutathione. The enzyme catalytic site includes a selenocysteine residue in which the selenium undergoes a redox cycle (Fig. **7**) involving the selenol (ESeH) as the active form that reduces hydrogen peroxides and organic peroxides [28, 29].

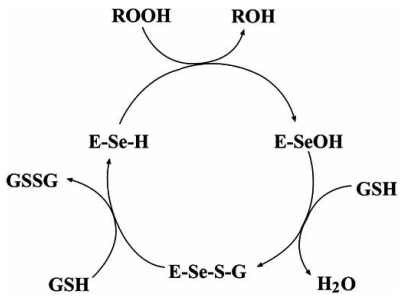
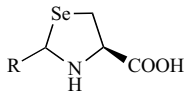


Fig. (7).

The selenol is oxidized to selenenic acid (ESeOH), which reacts with reduced glutathione (GSH) to form selenenyl sulfide adduct (ESeSG). A second glutathione then regenerates the active form of the enzyme by attacking the ESeSG to form the oxidized glutathione (GSSG). Thus, in the overall process, 2 equiv of glutathione are oxidized to the disulfide and water, while the hydroperoxide is reduced to the corresponding alcohol. Some organoselenium compounds have been shown to mimic the GPx activity *in vitro*.

A common feature of many of the implicated enzymes is their function in sequestering reactive oxygen species (ROS) and/or maintaining the cell and cellular components in their appropriate redox state. In addition some selenium compounds having peroxidase activity, glutathione S-transferases also play an important role in the detoxification of diverse electrophilic species, many of which arise during xenobiotic metabolism. High expression of some glutathione S-transferases has been shown to be protective against tumor development, and polymorphisms and other variations in glutathione S-transferase expression in humans have been linked to cancer incidence.

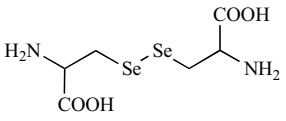
El-Sayed and Franklin [30, 31] reported a study into the induction of a protective hepatic enzyme for 2-substituted selenazolidine-4(*R*)-carboxylic acids (Fig. **8**). The results obtained increasingly appear to suggest that the effects observed may have a lot to do with the identities of the substituents in position-2 of the molecule.



Selenazolidines

Fig. (8).

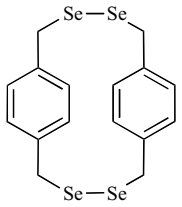
These authors [32] also carried out a comparative study of different forms of selenium. At equi-selenium doses, selenocystine (Fig. **9**), selenomethionine and methylselenocysteine provoked elevations in glutathione-S-transferase, but sodium selenite did not. However, selenite, selenocystine and selenomethionine were equally effective in preventing the decrease in glutathione peroxidase. These facts indicate and confirm that the biological effect can vary with the chemical nature of the compound administered and its metabolism.



Selenocystine

Fig. (9).

In relation to this aspect, Sohn *et al*. [33] investigated the metabolic pathway for *p*-XSC (Fig. **5**) in mice and rats. The detection of tetraselenocyclophane (TSC) (Fig. **10**) as a fecal metabolite in both led them to postulate that a glutathione conjugate (*p*-XSeSG) would be the primary metabolite of *p*-

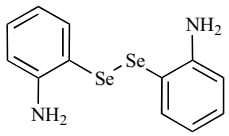


Tetraselenocyclophane

Fig. (10).

XSC through the following route: *p*-XSC to *p*-XSeSG to selenol (*p*-XSeH) to TSC. Complementary studies with other standards such as glutathione, cysteine and *N*-acetylcysteine conjugates of *p*-XSC confirmed the facile conversion of these compounds to TSC.

New experiments [34] with selenocystine, selenomethionine and 2-aminophenyldiselenide (Fig. **11**) established a novel mechanism for the prevention of oxidative DNA damage based on the requirement of selenium-metal coordination. In addition, the observed DNA damage inhibition by these selenium compounds contrasts with traditional GPx measurements for the antioxidant activity of selenium.



2-aminophenyldiselenide

Fig. (11).

Other mechanisms have been proposed for antioxidant action. Some data suggest that selenoproteins such as selenomethionine can decrease free radical generation [35]. Lu and Jiang [36] combined the role of selenoproteins and specific selenium metabolites for the expression of proteins [37] to account for cancer risk reduction. Recently, a study has been published [38] in which dietary antioxidants (tannic acid) and selenium compounds (selenomethionine) were shown to have a synergistic therapeutic effect against hepatocellular carcinoma.

Finally, some recent hypotheses suggest that the greatest anticarcinogenic potency for some selenium compounds is found for the +4 oxidation state [39] due to the direct oxidation of critical thiol-containing cellular substrates according the (Fig. **12**).

Fig. (12).

2.3. DNA Damage and Repair

DNA damage is an extremely common event in a cell's lifetime. The machinery that copies DNA when a cell divides is not 100% efficient. This means that tiny errors accumulate in our cells over our lifetimes. On top of this, the life-sustaining chemical reactions that occur naturally in our cells generate harmful by-products, and these can cause DNA damage. So merely 'being alive' can cause DNA damage and, potentially, cancer.

To make matters worse, there exist a number of environmental agents, that constantly damage the DNA in our cells. As a result of this continuous bombardment, some studies have estimated that the DNA in a single human cell gets damaged over ten times every day.

Thankfully, cells have evolved many complex mechanisms to detect and repair DNA damage, and most of the time a cell repairs its damaged DNA without a problem. However, just like the machinery that copies DNA, a cell's repair machinery is not 100% efficient and not every single error is corrected. So, as a back up, to prevent cancer occurring there are systems that cause a damaged cell to commit suicide if the DNA damage is too severe. DNA repair and apoptosis (cell suicide) have been the subject of huge amounts of research. Occasionally, despite all of these safety nets, the cell's repair machinery fails to correct the DNA damage, but doesn't trigger the cell's suicide apparatus. It is at this point that cancer can occur. In fact, some of the most harmful cancer-causing mutations are mutations in the genes that regulate DNA repair and apoptosis. There are two other important types of genes that, when mutated, can cause cancer – tumor suppressor genes and oncogenes. There are many other types of gene that can become mutated to make a cancer cell more 'successful' at surviving in the body. For example, some genes make proteins that allow the cell to travel down blood vessels, thus allowing a cancer cell to spread to other parts of the body. Others prevent damaged cells from being attacked by the body's immune system.

It has been known for a long time that some Se compounds have the potential to induce DNA damage [40]. Much of what is known about the DNA damage caused by Se is derived from bacterial and cell culture experiments. Studies of isolated hepatocytes as a model system indicated that SSe-induced DNA fragmentation was oxygen and redox cycle-dependent, thereby confirming the view that DNA damage by SSe in mammalian cells is also mediated by reactive oxygen species (ROS).

Earlier studies [41] showed that high selenite intake induces DNA single strand breaks (SSBs), reactive oxygen species (ROS), p53 Ser-15 phosphorylation and caspase-dependent and caspase-independent apoptosis, whereas a methylselenol precursor methylseleninic acid (MSeA) induces caspase-mediated apoptosis regardless of p53 status. Recently, Li [42] established that superoxide is a primary mediator for selenite in the induction of DNA-SSBs and p53 activation. Selenite [43] also acts as a potential modulator and enhancer of camptothecin-based anticancer therapy in nonovarian malignancies.

Other compounds proposed for the prevention of DNA damage are inorganic oxo compounds. This is the case for some selenates (Fig. **13**) [44,45] that may interact with alkylating carcinogens to protect DNA from alkylation. The explanation of the mechanism concerns the propensity to form oxo species in solution. Presumably the active anionic oxo species behave as potent nucleophiles towards electrophilic attack and these authors suggest that the design of new com-

Na_2SeO_4 $[(C_6H_5)_4P]_3(O_3SeOCH_2OSeO_3)(HSeO_4)$

Fig. (13).

pounds for cancer prevention should incorporate reactive oxo groups with high anionic charge density in order to accept alkylating toxins [45].

It has been observed that some foods, such as broccoli, are enriched with selenium during growth [46,47] and played an important role in the reduction of DNA single strand breaks associated with glutathione peroxidase activity in a study carried out with mouse liver hepatoma HEPA IcIc7 and C6 rat glial cells, respectively.

On the other hand, D-501036 [48], 2,5-bis(5-hydroxymethyl-2-selenienyl)-3-hydroxymethyl-*N*-methylpyrrole (Fig. **14**) has been investigated as a novel antineoplastic agent in the renal proximal tubule, normal bronchial epithelial and fibroblast cells and induces cell death associated with the DNA damage-mediated induction of ataxia telangiectasia-mutated activation.

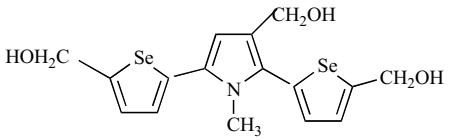


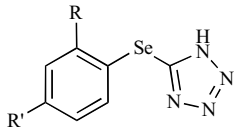
Fig. (14).

2.4. Selenium Compounds and Protein Kinases

Protein kinases are of particular interest in the treatment of human diseases because of their enzymatic activity and susceptibility to successful therapeutic targeting. The molecular targets that can be modulated by selenium include different kinases. For example, sodium selenite [49] provokes apoptosis in leukemia cells (NB4) mediated by protein kinase (ERK). A similar process occurs with selenoproteins [50] that regulate vascular endothelial growth factor (VEGF), a finding that correlates with a diminished invasive capacity of tumor cells. Other kinases studied include the kappa kinases. Sodium selenite and methylseleninic acid [51] inhibited NF-kappa B DNA binding and I kappa B kinase activation, I kappa B alpha phosphorylation and degradation induced by TNF-alpha. Park [52] later described how this same acid blocks tumor invasion by inhibiting pro matrix metalloproteinase-2 (MMP-2) activation mediated by suppression of membrane type-1 (MT1-MMP) expression, which is regulated by the nuclear factor-*k*B (NF-*k*B) signal. Previously, Jiang [53] reported that MeSeA reduced the phosphorylation level of mitogen-activated protein kinases (MAPKs) and suggested that a pool of methylselenol, an active metabolite from methylseleninic acid, might inhibit the expression of MMP-2 by vascular endothelial cells and of vascular endothelial growth factor (VEGF) by cancer epithelial cells. These two proteins are critical for angiogenesis and its regulation. The antiangiogenic activity may represent a novel mechanism for selenium compounds and this is supported by a recent study by Mousa [54], who provided evidence that selenium-derived compounds reverse the pro-angiogenesis effect of arsenic trioxide. Finally, a new role for kinases has been proposed by Hwang [55], who found that AMP-activated protein kinase (AMPK), which functions as a cellular energy sensor, mediates anticancer effects of selenium through a cyclooxygenase-2 (COX-2)/prostaglandin E signalling pathway.

2.5. Other Potentially Anticarcinogenic Selenium Compounds

Interest in selenium compounds has increased markedly in recent years. As a result, these compounds have become of interest to research groups as building blocks in the synthesis of selenium-containing heterocycles such as tetrazoles [56] (Fig. **15**), triazoles [57] (Fig. **16**), 1,3-selenazinanones [58] (Fig. **17**). Indeed, the anticarcinogenic activity of many examples of these compounds is under investigation.



R, R' = H; Cl; COOH; OCH_3; NO_2; CH_3.

Fig. (15).

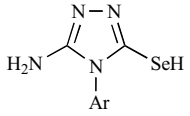


Fig. (16).

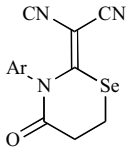


Fig. (17).

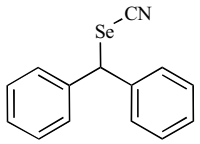
3. SELENIUM COMPOUNDS AND APOPTOSIS

3.1. Introduction

Programmed cell death (apoptosis) is a key tumor-suppressing mechanism and deregulated cell death pathways may lead to the development of cancer. As a result, the induction of tumor cell apoptosis [59-61] is the basis of many cancer therapies. Besides enabling malignant transformation and tumor progression, defects in apoptosis can result in resistance to cytotoxic cancer therapies. It is known that the targets of the apoptotic mechanism are complex, but a great deal of progress has been made in the delineation of the molecular pathways leading to apoptosis. Apoptosis may occur through an extrinsic pathway or an intrinsic or mitochondrial pathway. The extrinsic pathway is mediated by signals through death receptors of the tumor necrosis factor (TNF) superfamily and these death receptors are now one of the most attractive therapeutic targets in cancer research. In particular, DR5 and DR4, which are death receptors of the TNF-related apoptosis-inducing ligand (TRAIL or Apo2L), are interesting targets for antibody-based therapy. This is because TRAIL may also bind decoy receptors that could prevent TRAIL-mediated apoptosis, whereas the TRAIL ligand

itself selectively induces apoptosis in cancer cells. The mitochondrial apoptotic pathway is a highly regulated biological mechanism that determines cell fate and a wide range of apoptotic stimuli originating from other subcellular compartments (e.g. the nucleus, lysosomes, the endoplasmic reticulum, or the cytosol) converge on mitochondria where they favor mitochondrial membrane permeabilization (MMP) [62] which represents a crucial check-point in the cascade of events leading to cell death and cause the release of a number of apoptotic factors present in the mitochondrial intermembrane space. Upon permeabilization of the mitochondrial outer membrane (MOM), intermembrane space (IMS) proteins, that include caspase activators such as cytochrome c (Cyt c), Omi/HtrA2 (Omi stress-regulated endoprotease/ High temperature requirement protein A 2) and Smac/ DIABLO (second mitochondria-derived activator of caspase/ direct IAP binding protein with a low pI), as well as caspase-independent death effectors like apoptosis-inducing factor (AIF) and endonuclease G (EndoG), are released into the cytosol. Cyt c promotes the activation of the initiator caspase-9 in a direct fashion *via* the assembly of the apoptosome (together with the apoptosis protease activating factor-1, i.e. APAF-1, and ATP/dATP), while Omi/HtrA2 and Smac/ Diablo favor the caspase cascade indirectly, by antagonizing the activity of endogenous inhibitors of caspases, i.e. the inhibitor of apoptosis proteins (IAPs). AIF and EndoG translocate to the nucleus where they mediate chromatin condensation and large-scale DNA fragmentation, independently from caspases. The integrity of mitochondrial membranes is largely under the control of members of the Bcl-2 family. Bcl-2 family [63] includes both the pro and antiapoptotic members that play a central regulatory role in apoptosis. Currently, at least 20 members of the Bcl-2 family have been identified, all of which share at least one Bcl-2 homology (BH) domain. The family may be divided into three groups, one of which contains five anti-apoptotic proteins, Bcl-x_L, Bcl-w, A1, Mcl1, and Bcl-2 itself. Two further subfamilies are pro-apoptotic proteins; the Bax family has BH1-3 domains similar to those in Bcl-2, whereas the other pro-apoptotic proteins have only the BH3 domain. Commitment to apoptosis is typically governed by opposing factions of the Bcl-2 family of cytosolic proteins. Initiation of the proteolytic cascades requires assembly of certain caspase precursors on a scaffold protein, and the Bcl-2 family determines whether or not, this complex can form. Its pro-survival members can act by sequestering the scaffold protein and/or preventing the release of apoptogenic molecules like cytochrome-c from mitochondria. On contrary, its pro-apoptotic members act as sentinels for cellular damage as cytotoxic signals induce their translocation to the organelles where they bind to their prosurvival relatives, promote organellar damage and trigger apoptosis. Cytochrome-c is released either through channels created by integration of Bax and Bak in the mitochondrial membrane or following opening of the mitochondrial permeability transition (MPT) [64]. This channel is composed of proteins of both inner and outer mitochondrial membranes together with proteins of the intermembrane space, and its opening results in influx of ions, such as calcium, causing swelling of the mitochondria. This swelling produces breaks in the outer membrane, although the inner membrane remains intact, and cytochrome-c escapes through these outer membrane breaks. Other particular modality of cell death-associated with mitochondrial membrane permeabilization (MMP) is the permeability transition, which manifests by a loss of the inner transmembrane potential ($\delta\psi_m$), matrix swelling and the long-lasting opening of the permeability transition pore complex (PTPC) at the contact sites. The PTPC is composed by a minimum unit of three proteins, namely the voltage-dependent anion channel (VDAC) [65] in the mitochondrial outer membrane (MOM), the adenine nucleotide translocase (ANT) in the inner membrane (IM) and cyclophilin D in the matrix (CypD). These three proteins can interact within the contact site between the inner and the outer mitochondrial membranes (MIM and MOM, respectively). The minimum unit is regulated in its opening probability by the metabolic state of the cells, at the cytosol/intermembrane/matrix interphases, as well as by a panoply of additional proteins whose expression can be cell type-specific. At the level of the MOM, homo or hetero-oligomers composed of cytosolic proteins (e.g. Bax, Bid) and/or constitutive mitochondrial proteins (e.g. Bak, VDAC) or local ruptures have been proposed to mediate the release of intermembrane space proteins. Depending on the model, caspases and Bax/Bcl-2 family members can initiate, participate and/or regulate the mitochondrial phase of apoptosis. In addition, it appears that a large number of pro-apoptotic effectors, proteins or non-proteaceous in nature, can translocate to mitochondria and exert MMP-modulatory functions, thus influencing cell-fate at the level of this organelle. The two pathways, intrinsic and extrinsic converge in a common executor mechanism that involves activated proteases (caspases) and DNA endonucleases, which cleave regulatory and structural molecules and lead to cellular death.

Epidemiological studies, preclinical investigations and clinical intervention trials support the role of selenium compounds as potent cancer chemopreventive agents; the dose and the form of selenium are critical factors in cancer prevention. Induction of apoptosis and inhibition of cell proliferation are considered important cellular events that can account for the cancer preventive effects of selenium. Prior to apoptosis induction, selenium compounds alter the expression and/or activities of a number of cell cycle regulatory proteins, signalling molecules, proteases, mitochondrial associated factors, transcriptional factors, tumor suppressor genes and polyamine and glutathione levels. Depending on the form, selenium compounds can target separate pathways, although more research is needed to learn about disrupting different pathways that converge to apoptosis. Numerous selenium compounds are known to inhibit carcinogenesis in several animal models but not all of these have been examined for their efficacy in inducing apoptosis or vice versa in the corresponding target organ. Studies aimed at investigating the effects of selenium compounds on apoptosis in the target organ both *in vivo* and *in vitro* are limited. On the basis of information provided in this review, several novel synthetic organoselenium compounds need to be examined both *in vitro* and *in vivo* for their potential to induce apoptosis; such an investigation may provide improved and mechanism-based cancer chemoprevention as well as chemotherapeutic agents.



D enylmethylselenocyanate

Fig. (18).

In the following sections we describe selenium-containing structures that induce apoptosis and discuss their mechanisms of action and the potential for further development into novel therapeutics agents.

3.2. Apoptosis and Modulation of Caspases

Caspases are a conserved family of cysteine proteases that coordinate apoptosis, a process in which the cell is dismantled by targeting a panoply of proteins. The mammalian caspase family contains 14 members, with a subset participating in cellular demise and the remaining ones involved in the processing of pro-inflammatory cytokines. Defects in the expression or activity of these caspases are related to certain pathological conditions including neurodegenerative disorders, autoimmune diseases and cancer.

Some selenium compounds induce apoptosis through this mechanism. The synthetic compound diphenylmethyl selenocyanate (Fig. **18**) was evaluated [66] for its ability to induce apoptosis against 7,12-dimethylbenz[*a*]anthracene-croton oil and it was observed that caspase-3, which contributes in part to the process of cellular apoptosis to prevent further cellular differentiation, was elevated significantly. However, this compound had no modulatory effect on hepatocellular apoptosis [67] caused by acute doses of CCl_4.

2-(4-Methylphenyl)-1,3-selenazol-4-one (Fig. **19**) inhibits the proliferation and induces apoptosis [68] in a human ovarian cancer cell line (SKOV3) and an acute myelocytic leukemia cell line (HL-60), with caspase-3 activation in the latter cell line. These results suggest that the mechanism of apoptotic induction may differ between the two cell lines.

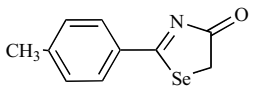


Fig. (19).

Methylseleninic acid (Fig. **2**) was found to enhance apoptosis induced by cancer therapeutic drugs in DU145 cells through MSA and some kinases that amplify the caspase-8 [69] activation cascade.

On the other hand, selenomethionine (Fig. **4**) – when combined with methioninase [70], which generates methylselenol from selenomethionine – induces caspase activation and loss of cell adherence in melanoma cells by altering integrin expression.

Several years ago, vitamin E and selenium were reported [71] to be effective in the prevention of prostate cancer. Structurally, vitamin E is related to phenolic compounds called tocopherols and tocotrienols. In order to gain more of an insight into the synergism between selenium and vitamin E, as well as to enhance the apoptotic properties of the latter compound, Vraka *et al*. [72] developed the synthesis of 2-phenylselenyl succinates from tocopherols and tocotrienols (Fig. **20**).

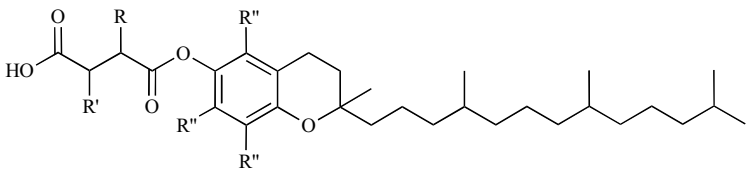
The efficacy of phenylselenyl succinate in inducing apoptosis was quantified and was associated with caspase-3 activation.

Finally, extensive studies on selenium have demonstrated the cytoprotective effects of this element on apoptosis cadmium [73] induction coupled with caspase-3 activation. Chan *et al*. [74] also demonstrated that CdSe-core quantum dots, a nanomaterial that has been shown to be useful as an alternative to fluorescent dyes for use in biological imaging, can induce apoptotic biochemical changes, including activation of caspase-9 and caspase-3.

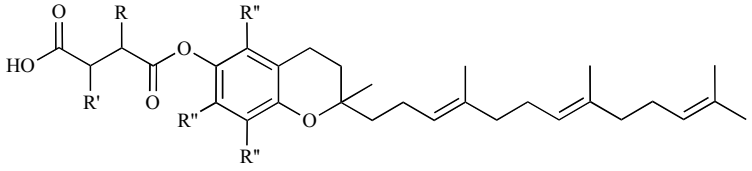
3.3. Apoptosis and Redox Process

The reactive oxygen species (ROS) can damage nucleic acids. The oxidative modification of DNA constitutes the fundamental molecular event in carcinogenesis and for this reason there is a great deal of interest in studying the involvement of ROS in that process. On the other hand, oxidative DNA damage-induced mutagenesis is widely hypothesized to be a frequent event in the normal human cell. An enormous amount of evidence suggests that ROS play an important role in the expansion and progression of tumor clones, which makes them a relevant class of carcinogens. ROS usually include [75] superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and the highly reactive by-product of H_2O_2, hydroxyl radicals ($^{\cdot}OH$), that are capable of reacting with and damaging DNA, proteins, and lipids. Aside from the damaging activity of ROS, low levels of intracellular ROS have also been identified as second messengers involved in a variety of signaling pathways and serve as transcription regulators. These diverse activities of intracellular ROS in initiating and/or amplifying death signals or in the regulation of apoptosis have been well studied and extensively reviewed. Recent data seem to suggest that $O_2^{\cdot -}$ may affect pathways involved in cell death and proliferation in a way distinct from H_2O_2.

On the other hand, cells in multicellular organisms are exposed to both endogenous oxidative stresses generated metabolically and to oxidative stresses that originate from neighboring cells and from other tissues. To protect themselves from oxidative stress [76], cells are equipped with reducing buffer systems (glutathione/GSH and thioredoxin/thioredoxin reductase) and have developed several enzymatic mechanisms against oxidants that include catalase, superoxide dismutase and glutathione peroxidase. Other major extrinsic defenses (from the diet) include ascorbic acid, beta-carotene and other carotenoids, and selenium. Recent evidence indicates that in addition to their antioxidant function, several of these redox species and systems are involved in the regulation of biological processes, including cellular signalling, transcription factor activity, and apoptosis in normal and cancer cells. The survival and overall well-being of the cell is dependent upon the balance between the activ-



R, R' = H, SePh; R" = H, CH_3 Tocopherol derivative



R, R' = H, SePh; R" = H, CH_3 Tocotrienol derivative

Fig. (20).

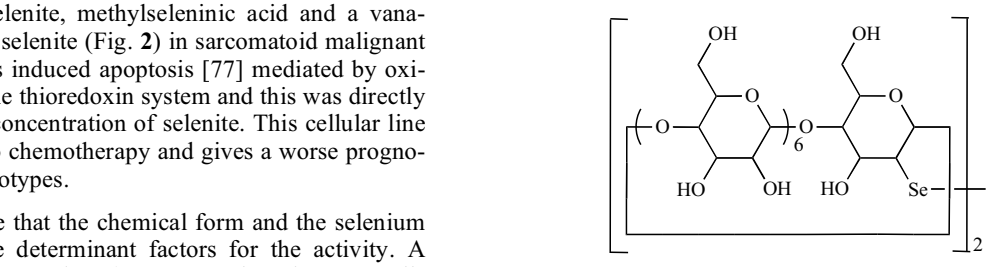
ity and the intracellular levels of these antioxidants as well as their interaction with various regulatory factors, including Ref-1, nuclear factor-kappaB, and activating protein-1. In addition, it is well known that reduced glutathione (GSH) is closely involved in the metabolism and bioactivity of Se.

We described, for the first time, inorganic compounds such as sodium selenite, methylseleninic acid and a vanadium salt. Sodium selenite (Fig. **2**) in sarcomatoid malignant mesothelioma cells induced apoptosis [77] mediated by oxidative stress and the thioredoxin system and this was directly dependent on the concentration of selenite. This cellular line is more resistant to chemotherapy and gives a worse prognosis than other phenotypes.

It is remarkable that the chemical form and the selenium oxidation state are determinant factors for the activity. A study on melanocytes, keratinocytes and melanoma cells showed the different effects of selenite and selenate [78] on apoptosis induction. Only selenite causes apoptosis and this compound has an effect on the thioredoxin reductase system. Li *et al*. [42] later reported that selenite exhibits distinct effects in two prostate cancer cells lines. LNCaP cells are more sensitive than PC3 cells to selenite-induced apoptosis and there is a rapid and high level production of superoxide in the former but only low levels in PC3.

Another inorganic selenium compound that causes apoptosis with the accumulation of reactive oxygen species (ROS) and the elevation of intracellular Ca^{2+} and Mg^{2+} is $Na_5SeV_5O_{18}.H_2O$ (NaSeVO) [79].

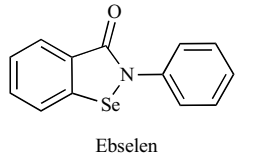
Among the organic compounds, Ebselen (2-phenyl-1,2-benzisoselenazol-3(2*H*)-one) (Fig. **21**) was revealed to possess strong antioxidant properties [80]. However, this compound does have some drawbacks, such as low glutathione peroxidase (GPX) activity and water insolubility. For this reason, Sun *et al*. [81] synthesized a novel GPX mimic (2-selenium-bridged β-cyclodextrin, 2-SeCD) (Fig. **22**) that has antioxidant efficiency superior to Ebselen and is non-toxic to NIH3T3 cells.



2-SeCD

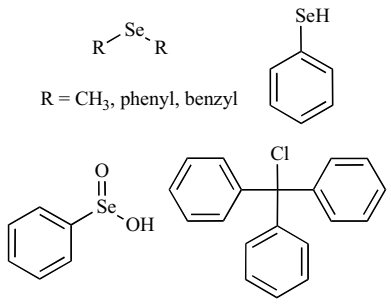
Fig. (22).

Recently, twenty seven selenium compounds [82] were tested for quinone reductase and glutathione-S-transferase activity in murine hepatoma cells. The most potent compounds were methylseleninic acid (Fig. **2**), Ebselen (Fig. **21**), some diselenides (dimethyl, diphenyl, dibenzyl), benzeneselenol, benzeneseleninic acid and triphenylselenonium chloride (Fig. **23**). The cell growth inhibition levels correlated with selenol metabolites.



Ebselen

Fig. (21).

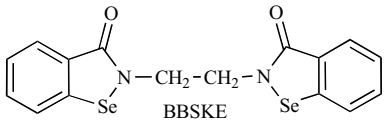


R = CH_3, phenyl, benzyl

Fig. (23).

3.4. Apoptosis and Cell Cycle

It is well known that there is a relationship between the cell cycle and apoptosis. Among the selenium compounds that are relevant in this area is selenomethionine (Fig. **4**), mainly because this is the major organic selenium compound found in the diet. Flow cytometry analysis showed [83] that selenomethionine induced G_2/M arrest in LNCaP by modulating transcript levels of genes involved in the cell cycle. Sodium selenite (Fig. **2**), in a study in NB4 cells, showed a concentration dependent effect on the cell cycle [84]. These findings confirm that selenium at a low concentration has a chemopreventive role against cancer, while at a high concentration it exerts an antitumor effect. Finally, the novel organoselenium compound 1,2-bis-[1,2-benzisoselenazolone-3(2*H*)-ketone]ethane (BBSKE) (Fig. **24**), which has shown an inhibitory effect on the growth of a variety of human cancer cells, provokes S phase arrest [85,86] accompanied by increases in the protein levels of cyclin A, E and p21 and decreases in levels of cyclin B1, D1 and Cdk4.



BBSKE

Fig. (24).

3.5. Apoptosis and Mitochondria

In this section only inorganic compounds will be discussed. It was observed that selenodioxide (SeO_2) and sodium selenite modulate apoptosis in a dose-dependent manner [87] in human oral squamous cell (HSC-3). These compounds do not lead to the generation of reactive oxygen species, but interfere in the mitochondrial redox equilibrium. Other studies with sodium selenite in osteoclast-like cells differentiated from RAW 264.7 cells [88] revealed that this compound promotes the generation of a superoxide anion and reduces the number of free thiol groups. In addition, selenite in human glioma cells induces cell death, disruption of the mitochondrial cristae, loss of mitochondrial membrane potential, degradation of proteins and leads to high levels of superoxide anions that trigger mitochondrial damage and mitophagy [89,90].

3.6. Apoptosis and Proteins

The protein kinase (PK) family of enzymes is a family of kinases that are involved in the transduction of signals for cell proliferation, differentiation, apoptosis and angiogenesis. Not surprisingly, disruption of PK regulation is implicated in tumorigenesis and drug resistance. Akt, also known as PKB, a cellular homolog of the oncogene product v-Akt encoded by AKT8 retrovirus, belongs to a family of serine/threonine kinases. This compound acts downstream of phosphoinositide 3-kinase (PI3-K) and plays a critical role in cell survival and oncogenesis. Over the last few years, the Akt pathway has become a major target due to its role as a signalling pathway in which modulation of substrates prevents apoptosis. The involvement of Akt in the cell survival pathway is a complex process that requires an extensive machinery for intracellular events. It has also been found that AKT plays an important role [91] in regulating apoptosis sensitivity of LNCaP cells and DU145 prostate cancer cells against methylseleninic acid. It was observed than MSA decreases phospo-Akt activity [92] in a time and concentration dependent manner. Too, inhibits P13-K activity and phosphoinositide-dependent kinase 1 (PDK1). The inhibition PI3-K could prevent tumor invasion and metastasis and the mechanism proposed for the inhibition is the interference as potent redox modulator [93] in the interchange global protein thiol/disulfide. However, these kinases did not significantly regulate apoptosis induced by sodium selenite in LNCaP. Recent evidence indicates that apoptosis induced by selenite in NB4 cells is mediated by the protein kinase ERK [49]. These findings support the differential involvement of these protein kinase pathways in regulating apoptosis induction by different forms of selenium. Other kinases that coordinate various extracellular signals to regulate cell proliferation, differentiation and cell survival are mitogen-activated protein kinases (MAPK), a family of structurally-related serine/threonine protein kinases [94]. Sodium selenite [95] in human acute promyelocitic leukemia (APL) cell line NB4 regulates signal transduction such as beta members of the MAPK family. Finally, another strategy that has been proposed for proapoptotic signal modulation is the coadministration of high doses of salicylates with organoselenium compounds [96] with the aim of decreasing NF-*k*B activity. This factor promotes transcription of a large number of proteins that inhibit both the intrinsic and extrinsic apoptosis pathways. In the case of BBSKE (Fig. **24**) a complementary mechanism for apoptosis has also been postulated and is based on the inhibition of the activity of thioredoxin reductase, leading to the accumulation of oxidated thioredoxin [97]; the change in the redox state of thioredoxin results in a decrease in the NF-*k*B DNA-binding activity, which in turn down-regulates the expression of some anti-apoptosis genes. Finally, other kinases implicated in some cancers are Src-kinases. Their activity can be regulated in several ways, being autophosphorylation site Y419 and negative regulatory phosphorilation site Y530 the most representatives [98]. Several protein tyrosine phosphatases (PTP) are capable of activating Src by dephosphorilating Y530 among them PTP1B that contribute to the progression in human colon cancer cells [98] and breast cancer [99,100]. Recent studies [100] have demonstrated that ablation of PTP1B has no consequences on breast tumorigenesis but have been obtaining promising results in lung tumor.

3.7. Apoptosis and p53 Regulation

Mutation of p53, a classical tumor suppressor, is frequently associated with oncogenesis. Cellular functions modulated by the p53 protein include DNA synthesis, DNA repair, cell cycle arrest, gene transcription, senescence and apoptosis. The most conserved function of the p53 protein is tumor suppression through the induction of apoptosis. Elevated expression of pro-apoptotic genes with promoters containing the p53 responsive element represents one mechanism whereby p53-dependent apoptosis is induced. Intracellular concentrations of p53 are increased markedly by stresses such as DNA damage, ionizing radiation, UV radiation, hypoxia, heat shock, growth factor withdrawal, oncogene activation and exposure to cytotoxic agents. Addition-

ally, the translocation of a specific polymorphic form of p53 from the cytosol to the mitochondria is associated with the induction of apoptosis. In response to the genotoxic insult, both transcription-dependent and transcription-independent cell death may be modulated concurrently by p53 with the resultant amplification of the apoptotic signal. Among the selenium compounds, selenomethionine (Fig. **4**) modulates p53 activity by causing redox regulation of key p53 cysteine residues [101]. In addition, treatment with methylseleninic acid gave rise to phosphorylation of one or more p53 threonine residues, but did not affect any known serine phosphorylation sites [101]. On the other hand, selenium dioxide [102] in leukemia cell lines and sodium selenite [103] in cultured cortical neurones showed up-regulation for p53 and superoxide production mediated by p53 [104] in LNCaP. Selenomethionine [105], in colon cancer cells, exerts p53-dependent growth inhibitory effects by inducing G_2/M cell cycle arrest. Recently, a selenophene derivative has emerged [106] that induce apoptosis through activation of p53 in a time-dependent manner. Additionally, p53 was found to be translocated from the cytosol to the mitochondria in response to drug treatment for 12 h.

CONCLUSIONS

It is clear from the studies discussed above that the use of selenium compounds does have effects on growth, cell cycle and apoptosis and that such compounds offer great promise as anticancer and apoptotic agents. As a result of these studies, selenium derivatives are rapidly emerging as valid chemotherapeutic agents. However, various organic and inorganic selenium compounds used in some studies have produced variables results when are tested in animal models and human subjects and more investigations are urgently needed in order to conclude the maximum safety in their utilization. This mini-review covers mainly the general chemical structures that incorporate selenium published since 2005 with the aim of reviewing the most recent articles in this field. Although several possible mechanisms have been proposed to explain the anticancer and apoptotic properties of selenium compounds [107], the results described here suggest the following preliminary considerations:

1- The chemical form is determinant for the activity. The methylated forms, for example, methylselenocysteine, methylseleninic acid or selenomethionine, require metabolism to methylselenol for anticarcinogenic activity.

2- The effect of some selenium compounds mainly depends upon the dose and the oxidation state of selenium. For inorganic selenium compounds +4 show the highest anticarcinogenic properties and for organic selenium compounds mainly +2.

3- The existence of diverse responses for the same chemical structure suggest several mechanisms of action. For example, sodium selenite induces apoptosis by redox processes, p53 regulation and a protein kinase-mediated mechanism amongst others. The expectation of a broad therapeutic benefit from agents that target only one member of either pathway may be overly simplistic due to the complex interrelated network governing apoptosis.

4- Experimental evidence shows that molecular symmetry, as a broad concept, could be a positive factor for cancer prevention and apoptosis (sodium selenite, methylseleninic acid, *p*-XSC, *p*-XMS, selenocystine, tetraselenocyclophane, 2-aminophenyldiselenide, diphenylmethylselenocyanate, 2-SeCD, diaryl and dialkylselenides, BBSKE). The importance of molecular symmetry in cytotoxic and proapoptotic activities was reported by us [108] in 2006. Recently, we described [109] a new series of symmetrical potent organoselenium compounds as cytotoxics in prostate cancer cells.

Nevertheless, this class of compound offers a great deal of promise to significantly broaden the horizon of modern apoptosis and anticancer drug discovery. Animal data, epidemiological data, and intervention trials have shown a clear role for selenium derivatives in both prevention of specific cancers and antitumorigenic effects in postinitiation phases of cancer so as apoptosis induction. Accordingly, there has been substantial interest directed toward the synthesis of selenium-containing derivatives in recent years that could be used as cytotoxic, cancer chemopreventive and apoptotic agents. However, a great deal of further research is needed to unravel the precise manner in which selenium compounds act.

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