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Ebselen, a promising antioxidant drug: mechanisms of action and targets of biological pathways

Gajendra Kumar Azad · Raghuvir S. Tomar

Abstract Ebselen, an organoselenium compound, mimics glutathione peroxidase activity. It is a multifunctional compound, which catalyzes several essential reactions for the protection of cellular components from oxidative and free radical damage. Based on a number of in vitro and in vivo studies, various mechanisms are proposed to understand the biomedical actions of ebselen in health and diseases. It modulates metallo-proteins, enzymatic cofactors, gene expression, epigenetics, antioxidant defenses and immune systems. Owing to these properties, ebselen is currently under clinical trials for the prevention and treatment of various disorders such as cardiovascular diseases, arthritis, stroke, atherosclerosis, and cancer. A few ebselen-based pharmaceutical agents are under extensive investigation. As ebselen has been shown to have significant cellular toxicity, appropriate studies are needed to redesign the ebselen-based therapy for clinical trials. This review summarizes current understanding of the biochemical and molecular properties, and pharmacological applications of ebselen and future directions in this area of research.

Keywords Ebselen · Organoselenium compounds · Redox biology · Antioxidant · Enzyme mimic

Introduction

Ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one) is an organoselenium compound (Fig. 1), chemically is an electrophile [1]. The general mechanism of action of ebselen is through reactions with specific cysteine thiol groups in proteins [2, 3]. Ebselen (EBS) catalyzes the reduction of reactive oxygen species (ROS) in a manner similar to glutathione peroxidase (GPx), which operates by a simple, three-step mechanism involving changes in the oxidation state of the active site (SeCys) residue (Fig. 2). ROS oxidize the resting state selenol (EBS-SeH) to selenenic acid (EBS-SeOH), which is reduced to the active selenol by glutathione (GSH) through a selenenyl sulfide intermediate (EBS-SeSG) [4, 5].

Ebselen also reacts with the thioredoxin (Trx) system [3]. Ebselen is an excellent substrate for the mammalian thioredoxin reductase (TrxR) and acts as a highly efficient oxidant of the reduced Trx, and catalyzes the hydrogen peroxide (H_2O_2) reductase activity of TxR. Similarly, ebselen has also been shown to act as a dehydroascorbic acid (DHA) reductase mimetic [6]. A considerable extent of the pharmacological activity of ebselen is attributed to its antioxidant action. Recent animal studies have demonstrated the anti-inflammatory activities of ebselen in a variety of experimental animal models [7, 8]. Due to its antioxidant behavior, it exhibits anti-lipoperoxidative, anti-atherosclerotic, anti-thrombotic, and cytoprotective properties [9–15]. Ebselen is a lipid-soluble compound, and therefore readily enters the cell. Ebselen potently inhibits lipid peroxidation through a GPx-like action, as it has the ability to oxidize glutathione thiol (GSH) and reduce hydrogen peroxide to water [16–19]. Moreover, ebselen has a strong protective ability against cytotoxicity and DNA damage caused by the reactive oxygen species (ROS) generated during various cellular processes [20]. ROS, including H_2O_2, superoxide radical ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$), and the reactive nitrogen species (RNS) such as peroxynitrite ($ONOO^-$) have been shown to increase upon

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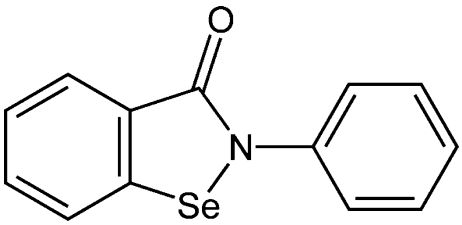

Fig. 1 Chemical structure of Ebselen (2-Phenyl-1,2-benzoselenazol-3-one)

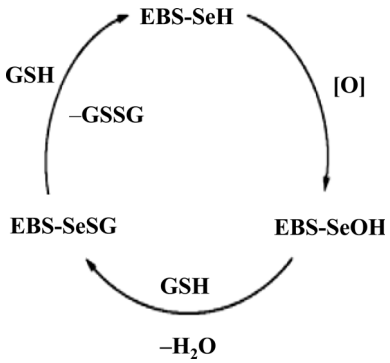

Fig. 2 Catalytic mechanism of action of ebselen. Ebselen (*EBS*) catalyzes the reduction of reactive oxygen species (*ROS*), which operates by a simple, three-step mechanism involving changes in the oxidation state of the active site Se molecule. ROS oxidize the resting state selenol (*EBS-SeH*) to selenenic acid (*EBS-SeOH*), which is reduced to the active selenol by glutathione (*GSH*) through a selenenyl sulfide intermediate (*EBS-SeSG*)

ischemia and reperfusion damage [17]. Ebselen rapidly reacts with peroxynitrite and other free radicals generated during ischemia or reperfusion [21–23] and protects against the deleterious effects of these ROS and RNS [5, 8, 24–28]. Due to this wide range of effects, ebselen has been investigated for the treatment of various ailments such as arthritis, stroke, organ transplantation, anti-asthamatic, ozone toxicity, diabetes-related atherosclerosis and nephropathy, and as an inhibitor of the neuronal demyelination [11, 29–32].

Contrary to the beneficial effects shown by ebselen, it also causes cellular toxicity. Based on various studies it is known to cause deterioration of the mitochondrial function by stimulating Ca^{2+} release from the mitochondria through the NAD^{+} hydrolysis-dependent mechanism [15, 33, 34]. Furthermore, high concentrations of ebselen induce the necrotic cell death of Sp2/0-Ag14 hybridoma cells [35]. It also causes genotoxicity, induction of DNA damage in V79 cells [36], and apoptosis in HepG2 cells [37]. Recently, it has been that ebselen can also induce DNA damage in the yeast (*Saccharomyces cerevisiae)* leading to activation of the checkpoint kinase proteins and derepression of DNA damage response genes [38]. Ebselen also inhibits the DNA binding mechanisms of the cysteine- and zinc-containing transcription factors including TFIIIA, Sp1, and NF-kappa B [39]. Ebselen reacts with metallothionein (MT) and other biological zinc/sulfur coordination sites to release zinc. As a result, zinc finger motifs are highly reactive towards this compound, possibly affecting the gene expression, DNA repair and, therefore, genomic stability [40].

Taken together, ebselen targets a wide variety of proteins and modulates several biological processes, leading to both beneficial and harmful actions in the biological systems. Ebselen is being used in clinical trials for the treatment of various diseases. The outcome of these studies may provide additional insights into the mechanisms underlying the biological properties of ebselen. The focus of this review is to summarize the biological/biochemical and molecular pathways targeted by this antioxidant molecule.

Ebselen localization inside the cell

Experimentally, it has been demonstrated that upon administration into the bloodstream, extracellular ebselen is transported as an albumin complex [41] bound to the reactive cysteine residue in albumin [42]. It is difficult to track the intracellular movement of ebselen within the cell because it is not fluorescent. The attachment of tags or reporter groups may fundamentally change the physiochemical properties of ebselen. However, ebselen does contain one potentially useful spectroscopic marker, in that it integrates an organoselenium moiety within its structure. As a trace element, selenium (Se) is present in cultured cells in extremely low concentrations. Therefore, the Se content of the ebselen-treated cells would be expected to be much higher than that of cells with normal background Se levels. Based on this selective increase of Se, it is anticipated that the analysis of cellular Se distribution would provide information on the intracellular localization and concentration of ebselen. Recently, Aitken et al. [43] have adopted this approach to analyze the intracellular localization of ebselen by using synchrotron radiation-induced X-ray emission (SRIXE) spectroscopy. Based on their results ebselen uptake appears to peak after 2 h, following which the cellular Se content declines, probably due to metabolic complexation and drug efflux. Analysis of the elemental maps suggests that ebselen or its metabolites immediately localizes in the ER following uptake and remains predominantly partitioned within this organelle for the 4 h of the experimental duration [43].

Several reports indicate that the mitochondria are also potential targets of ebselen [44–46]. In rat liver, the effect of ebselen on the electron transport chain has been studied.

Table 1 List of the proteins whose activity is inhibited by ebselen

S. No.	Enzyme/protein	Action of ebselen	References
1	JMJD2A	Disrupts Zn binding site	[63]
2	TFIIIA and xeroderma pigmentosum group A	Disrupts Zn finger motif	[39, 64]
3	Alcohol dehydrogenase and metallothionine (MT)	Disrupts Zn finger motif	[40]
4	Fpg (formamidopyrimidine-DNA glycosylase)	Disrupts Zn finger motif	[64]
5	Lipoxygenases	Alteration of the iron ligand sphere	[50, 51]
6	NO synthases	Reacts with its critical thiol group	[52]
7	NADPH oxidases (Nox)	Inhibits assembly of the Nox2 regulatory subunits	[53]
8	Horseradish peroxidase	Interferes with sulfhydryl groups	[55]
9	H+/K(+)-ATPase	Interferes with sulfhydryl groups	[54]
10	Lactate dehydrogenase	Interacts with critical thiol groups	[56]
11	P50 subunit of NF-kB and Sp1	Disrupts its Zn finger motif	[39]
12	Heme enzyme indoleamine 2,3-dioxygenase (IDO)	reacts with multiple cysteine residues and disrupts its active site	[58]
13	Na+, K+-ATPase	Interacts with critical thiol groups of this enzyme	[57]
14	Inositol Monophosphatase (IMPase)	Covalent and irreversible inhibition	[130]
15	*P. falciparum* Hexokinase (PfHK)	Non-covalent interaction with HK	[153]
16	*Pseudomonas aeruginosa* Diguanylate cyclases	Reacts with its critical thiol group	[150]
17	divalent metal transporter-1 (DMT1)	Noncovalent interaction	[102, 103]

At low micromolar concentrations, ebselen inhibits the flow of reducing equivalents from NADPH-cytochrome P450 reductase to both of its electron acceptors, cytochrome P450 and cytochrome *c* [47]. In addition, ebselen has been shown to protect the mitochondrial membranes against peroxidative reactions in the liver mitochondria isolated from glutathione-depleted rats [48]. Furthermore, ebselen significantly prevents the mitochondrial depolarization in ischemic astrocytes [25]. Another report suggests that depending on the concentration used, ebselen exert deleterious actions on astrocyte physiology by altering the permeability of the mitochondrial membrane as well as calcium homeostasis [49]. Taken together, these results indicates that the ER and mitochondria are the most preferred intracellular targets of ebselen and, presumably, the pharmacological activity of this compound might be attributed to its ability to modulate the function of these two organelles. Once inside the cell, ebselen covalently and non-covalently interacts with several biomolecules. In the next section, we have described some of the well-established molecular targets of ebselen.

Intracellular targets of ebselen

In the last few years, many analytical methods (e.g., X-ray diffraction, high performance liquid chromatography, nuclear magnetic resonance, infrared, circular dichroism, and ultraviolet–visible (UV/Vis) spectroscopies) have been utilized to characterize the drug-protein systems. These techniques have been used to show that ebselen reacts with the thiols to form stable selenenosulfide bonds [50]. Moreover, ebselen appears to act on multiple pathways and protein targets, and its mode of action in cells is pleiotropic. The reactivity of ebselen with the protein thiols makes it a potent modulator for proteins that require cysteine for normal function. Ebselen inhibits a number of enzymes involved in various biological processes, such as lipoxygenases [51, 52], nitric oxide synthases (NOS) [53], NADPH oxidases [54], H^+-K^+-ATPase [55], horseradish peroxidase [56], glutamate dehydrogense [57], lactate dehydrogenase [58], and many others listed in Table 1. In most cases, ebselen targets critical thiol residues of enzymes, and inhibition can be reversed by the addition of reducing agents such as dithiothreitol (DTT) [53, 55, 59–61].

The zinc-finger (ZF) domain is another ebselen target. ZF transcription factors contain cysteine and histidine residues (characteristically Cys4, Cys3His, or Cys2His2) that tetrahedrally coordinate Zn^{2+} ions to ensure appropriate folding of the ZF tertiary structure, which is required for the biological recognition [62, 63]. The Zn^{2+} ion coordinates to a ZF in its reduced state, in which all cysteine residues are in the thiolate form. Alterations in the oxidation state of cysteine cause the release of Zn^{2+}, leading to the disruption of tertiary structure and ultimately the loss of ZF function. Ebselen inhibits the function of several ZF-containing proteins by a similar mechanism [64]. For example, JMJD2A, a 2-oxoglutarate- dependent N(epsilon)-methyl lysine histone demethylase, is inhibited by the disruption of its Zn-binding site [65]. Ebselen reacts with metallothionein (MT) and alcohol dehydrogenase to

release zinc [40]. Ebselen also inhibits the DNA binding mechanisms of several ZF-containing proteins, including TFIIIA (transcription factor IIIA), DNA binding domain (DBD) of the NF-κB mediated transcription factor Sp1 [39], formamidopyrimidine DNA glycosylase (Fpg) [66], xeroderma pigmentosum group A protein (XPA), and NF-κB affecting the expression of downstream genes [39, 66]. Apart from its inhibitory effect on many DNA binding proteins, ebselen can also induce transcription of the anti-oxidant response element (ARE) [67] and nuclear factor erythroid 2-related factor 2 [68, 69].

Due to its interaction with several classes of biomolecules, it is likely that this molecule will influence a broad range of biological processes. In the next section, we will describe some of these.

Biological pathways affected by ebselen

Recent studies have illustrated that ebselen is a highly pleiotropic molecule that interacts with several molecular targets inside the cell. Ebselen may alter the activity of target proteins by direct binding or regulate the protein function indirectly.

Apoptosis

Apoptosis occurs normally during development and aging, and functions as a homeostatic mechanism to maintain cell populations in tissues [70]. At low doses, a variety of injurious stimuli such as heat, radiation, hypoxia, and cytotoxic anticancer drugs can induce apoptosis [71, 72]. Ebselen is known to prevent apoptosis that might occur due to injury or exposure to toxic agents. For example, it prevents apoptosis induced due to reperfusion of ischemic tissues by reducing the oxidative stress associated with ischemia/reperfusion [73–76]. The alkylating agent nitrogen mustard (HN2) and ionizing radiation can cause apoptosis through the production of oxygen free radicals. Ebselen provides efficient protection against HN2-induced cell death in lymphocytes, and can prevent cellular damage caused by the alkylating agents [77]. Ebselen is equally effective against ionizing radiation. Upon exposure to γ-irradiation, there is a distinct difference between untreated cells and cells pretreated with ebselen with respect to viability, cellular redox status, and oxidative damage [78]. Treatment of cells with ebselen reduces peroxide levels, protecting the thymocytes from radiation-induced apoptosis produced during and after gamma-irradiation [79]. Ebselen attenuate oxidative stress-induced apoptosis by scavenging free radicals in PC12 cell lines [80].

The mechanism through which ebselen prevents apoptotic events remains unclear. One study showed that in response to γ-irradiation, ebselen effectively suppresses programmed cell death by decreasing the apoptotic events, such as the caspase activation, increased levels of anti-apoptotic molecules (Bcl-2), and decreased levels of proapoptotic molecules (Bax), presumably via the preservation of redox status [78]. Similarly, it also attenuates the H_2O_2-induced cell death through the inhibition of signaling pathways mediated by p38 MAP kinase (p38-MAPK) [81, 82]. The neuroprotective effects of ebselen at low doses are associated with the increased expression of Bcl-2 and inhibition of Bax proteins [83]. Furthermore, ebselen potently inhibits cytochrome *c* release and caspase-3 activation in differentiated PC12 cells in response to nitric oxide (NO)-induced apoptosis. Ebselen also blocks the activation of apoptosis signal-regulating kinase 1 (ASK1) and c-Jun N-terminal protein kinase (JNK) [84]. In addition, it also activate p44/42 MAPK and inhibit the down-regulation of Bcl-2 in SNP-treated PC12 cells [82]. Similar effects have been observed in a mouse model upon ebselen treatment. Furthermore, it has been demonstrated that ebselen attenuates ischemic neuronal apoptosis by inhibiting cytochrome *c* release in ICR mice [28].

Although most studies show that ebselen inhibits apoptosis under different experimental conditions, the mode of action is primarily through the interaction with thiol-containing proteins. Interestingly, ebselen induces apoptosis in HepG2 cells in a dose- and time-dependent manner. Evidence suggests that ebselen exposure causes rapid depletion of intracellular thiols leading to apoptotic cell death [37].

Genomic Integrity

DNA is constantly damaged by exogenous and endogenous factors. One of the main causes of DNA damage is the production of ROS, which are highly reactive molecules that are constantly generated due to metabolic activities or exposure to environmental factors [85, 86]. ROS can cause single- or double-stranded breaks. Ebselen offeres strong protection against ROS-mediated cytotoxicity and DNA damage [22, 87, 88]. Ebselen exhibits protective actions against H_2O_2-induced cytotoxicity and DNA damage in HepG2 cells [87]. In vitro, ebselen efficiently protects against peroxynitrite-induced DNA single-strand breaks through free radical scavenging [89, 90]. Low concentrations of ebselen (5–10 μM) do not induce DNA damage in V79 cells and budding yeast, while the same concentrations diminish the extent of DNA damage induced by H_2O_2 [36]. Ebselen exhibits DNA protective effects against several toxic compounds. For example, it attenuates the cyclophosphamide-induced DNA damage in mice [91], and it also significantly reduces formation of the hepatic aflatoxin

B1 (AFB1)–DNA adducts and 8-hydroxydeoxyguanosine following administration of AFB1, a potent hepatocarcinogen, in a rat model [92].

However, at higher doses, ebselen acts as genotoxic agent and induces DNA damage [36]. Significant growth arrest have been reported in yeast cells exposed to ebselen [93]. Further, it has been demonstrated that ebselen causes the derepression of DNA repair genes through the activation of the Mec1-Rad53-Dun1 kinase pathway [38], this kinase pathway regulates DNA damage response in yeast cells [94]. We have noted that ZF (zinc finger) motifs are highly reactive towards oxidizing selenium compounds including ebselen. Potentially, ebselen-induced disruption of the ZF motif adversely affects gene expression, DNA repair and, therefore, genomic stability [66].

Immune system

Ebselen exhibits pleiotropic modes of action as it targets different biological pathways. Ebselen is known as a modest cytokine inducer or immunostimulant [95]. Ebselen exposure has been reported to induce several interleukins including interleukin-1 (IL-1), IL-6, IL-10, and IL-18. Furthermore, ebselen stimulates production of the interferon (IFN)-gamma, tumor necrosis factor (TNF)-α, intercellular adhesion molecule (ICAM)-1, affecting immune cell adhesion and migration and contributing to its anti-inflammatory properties [96–99]. Conversely, ebselen has been shown to inhibit the TNF-α production and mRNA expression in rat primary Kupffer cells. In addition, ebselen neutralizes the liver injury induced by the superantigen staphylococcal enterotoxin B [96], suggesting that ebselen has an immunomodulatory function. Ebselen also inhibited inhibits the lipopolysaccharide (LPS)-induced phosphorylation of JNKs (c-Jun amino-terminal kinases), leading to decreased transcription of TNF-α [100].

Ebselen administration reduces the serum levels of TNF-α, IL-18/IGIF (Interferon-gamma-inducing factor), and interferon (IFN)-ϒ after LPS administration in mice [101]. It has also been reported that ebselen attenuates liver injury induced by TNF-α and enhances the release of IL-10 [79]. Similarly, treatment of rats with ebselen lowers plasma IL-6 levels in glial cells, leading to decreased levels of heme oxygenase-1, an inducible heat shock protein [97]. Ebselen also inhibits ICAM-1 expression in leukocytes [102] and decreases TNF-α levels, leading to suppression of bronchiolar inflammation [103]. Additionally, ebselen is found to inhibit the adhesion of polymorphonuclear leukocytes (PMNL) to IL-1-activated endothelium and to inhibit the transendothelial PMNL migration [104]. Ebselen is equally effective as a suitable anti-inflammatory agent in ocular tissues [105]. One interesting study show that ebselen treatment enhances the viability of bone marrow-derived cells (BM-DCs) during transplantation by decreasing oxidative stress during this procedure [106]. Lung function in the ebselen-treated rats is also significantly improved at 24 h after transplantation [26].

Collectively, the results of the above studies suggest that ebselen modulates the host immune system and exhibits anti-inflammatory effects in response to various stimuli.

Cellular transport

Different genetic screening assays have shown that ebselen inhibits activity of the divalent metal transporter 1 (DMT1) [107–110]. DMT1, a proton-coupled metal-ion transport protein expressed in neurons is known to actively transport several different divalent cations such as Fe^{2+}, Zn^{2+}, Mn^{2+}, Co^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+}, and Pb^{2+} [111, 112]. Interestingly, ebselen has been found to be specific for inhibiting uptake of iron but not manganese [109]. Surprisingly, DTT supplementation fails to reverse inhibition of DMT1-mediated iron uptake, suggesting that ebselen does not block DMT1 transporter function by direct modification of protein thiols. It has been proposed that ebselen might influence the activity of specific factors involved in the intracellular targeting of iron [109]. Recently, it has been demonstrated that DMT1 inhibition by ebselen leads to reduction in ferrous iron-induced activation of CDK5 (cyclin-dependent kinase 5) and GSK3β (glycogen synthase kinase 3 beta) in human neuroblastoma SH-SY5Y cells [113]. However, ebselen not only interferes with iron metabolism in cells, but also affects the intracellular calcium homeostasis. One study shows that ebselen hampers an agonist-triggered increase in intracellular calcium by inhibiting inositol 1,4,5-trisphosphate (IP_3)-induced calcium release [114, 115]. Ebselen has also been shown to increase free cytosolic Ca^{2+} concentrations in rat hippocampal astrocytes [116]. Additionally, ebselen has been found to degrade mitochondrial function by stimulating Ca^{2+} release from mitochondria [33]. As calcium is involved in several signaling pathways, these results suggest that alteration of calcium homeostasis by ebselen might affect the downstream pathways. For example, ebselen inhibits the kinase activity of partially purified Ca^{2+}- and phospholipid-dependent protein kinase C [117]. Taken together, these studies indicate that ebselen targets iron and calcium homeostasis in the cells by modulating the transporter and storage of these ions.

Thus far, we have described the molecular targets and biological pathways targeted by ebselen. A considerable body of research has revealed that ebselen has a surprisingly wide range of beneficial properties, including anti-inflammatory, antioxidant, anti-mutagenic, and many more as shown in Fig. 3. These activities have been demonstrated both in cultured cells and in animal models. In the next section, we will explain the therapeutic uses of ebselen.

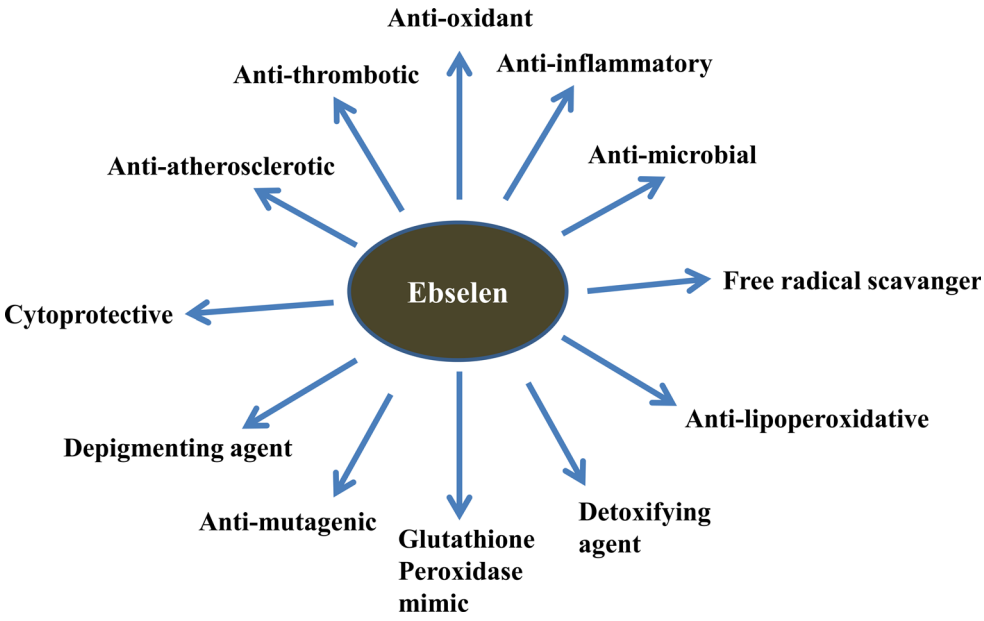

Fig. 3 Schematic representation of biological activities displayed by ebselen. Based on the studies reported, ebselen is a bioactive molecule, which exhibits myriads of properties as described in the figure

Ebselen in therapeutics

ROS such as superoxide anion, hydroxyl radicals, and hydrogen peroxide are responsible for lipid peroxidation, protein oxidation, and DNA damage. These effects are deleterious to cells because they accelerate cell senescence and aging, and lead to various age-related diseases, diabetes and its associated disorders, neurologic disorders including stroke, Parkinson's disease, Alzheimer's disease, and diseases associated with cartilage degradation [118–120]. Oxidative stress resulting from ROS production is also one of the main causes of apoptosis [121, 122]. Antioxidant compounds and enzymes that have the potential to scavenge free radicals are of great pharmacological importance. Ebselen appears to be a promising drug because of the multiplicity of its mechanisms for inhibiting free radical-induced injury, coupled with its lack of side effects, good blood–brain permeability, and rapid absorption following oral administration [123]. A summary of pharmacological applications of ebselen are shown in Table 2.

Neuroprotective effects of ebselen

Various studies have demonstrated that ebselen attenuates neuronal cell death induced by ischemia/reperfusion [74, 75]. It has been reported that ebselen affects the morphological structure of neurons, and reduces the degree of oxidative stress [124] through its GPx(glutathione peroxidase)-like and anti-lipoperoxidative activities [125]. Direct interaction of ebselen with the glutamatergic system has been shown in rats [13], suggesting that ebselen reduces glutamate-induced oxidative stress in different regions of the rat brain [126]. The mechanism of action for this effect has not been established, but preliminary studies suggest that ebselen protects neurons from ischemic damage via control of the expression of γ-aminobutyric acid (GABA) shunt enzymes to supply the tricarboxylic acid cycle (TCA) cycle [24]. Ebselen also significantly inhibits acetylcholinesterase (AChE) activity [127], demonstrating its involvement in the cholinergic system. In rodent models, ebselen has been shown to provide significant protection against ischemic damage in both gray and white matter [8] and the ventroposterior nucleus (VPN) of the brain [128]. Additionally, the administration of ebselen in gerbils significantly reduces neuronal death induced by ischemia/reperfusion in the hippocampal CA1 region [74].

Lithium is the most effective mood-stabilizing drug for the treatment of bipolar disorder [129]. Unfortunately, lithium usage is associated with a high degree of toxicity and has many undesirable side effects at therapeutic doses [130]. Lithium is active in biopolar disorder primarily by inhibiting inositol monophosphatase (IMPase) [131, 132]. Recently, it has been reported that ebselen inhibits IMPase and acts as a lithium mimetic in mouse models of bipolar disorder [133]. It was also found that inhibition of IMPase by ebselen is covalent and irreversible. This offers several advantages, since irreversible inhibition cannot be overcome by accumulation of substrate, prolonging the activity of ebselen and increasing its selectivity for IMPase [133]. Therefore, ebselen represents an alternative to lithium that might serve as a safer treatment for bipolar disorder.

Ebselen as a potential treatment for diabetes-related disorders

The ability of ebselen to mimic glutathione peroxidase (GPx) activity makes it a potential drug for the treatment of diabetes-

Table 2 List showing potential use of ebselen in Pharmacology

S. No.	Complications	Ebselen mode of action	Reference
1	Eye problems due to alcohol	Decreases neurotoxicity induced by ethanol	[31]
2	Brain disorders associated with demyelinating events	Inhibit AChE activity	[122]
4	Arthritic joints	Blocks cartilage proteoglycan breakdown	[30]
5	Acute lung injuries(ozone exposure)	Modulates the oxidant-related inflammatory process	[29]
6	MeHg-induced cytotoxicity	Suppresses oxidative stress	[141, 142]
7	Nitrogen mustards toxicity	Reduces membrane damage	[148, 149]
8	Cadmium-induced testicular damage	Reduces the oxidative stress	[143]
9	Cisplatin-induced nephrotoxicity	Reduces the oxidative stress	[144]
10	Inflammation-associated carcinogenesis	regulates expression of pro-inflammatory mediators	[7, 74]
11	Mn–induced toxicity		[146]
12	Diabetes-related atherosclerosis and nephropathy	Effectively repletes the lack of GPx1	[11]
13	Vascular complications in patients with Diabetes-mellitus (DM)	Scavanges free radicals	[134]
14	Opportunistic pathogens such as *Cryptococcus neoformans* and *Candida albicans* infection	Block the activity of Pma1p	[149, 155]
15	Anti-malarial activity	Selective inhibition of plasmodia	[154]
16	Anti-asthma activity	Suppression of oxidant formation and iNOS induction	[32]
18	Anti-thrombotic agent	Reduces oxidative stress	[12]
19	Chronic iron overload	Reduces oxidative stress	[140]
20	Lung oedema	Reduces BALTNF-a and ET-1 levels	[98]
21	Oxidant stress-associated thrombotic events	Reduces blood cell aggregate formation and vessel occlusion	[12]

related disorders associated with reduced GPx levels. Ebselen effectively compensates for the lack of GPx and acts as an effective therapeutic molecule for the treatment of diabetes-related atherosclerosis and nephropathy [11]. In diabetic rats, ebselen considerably reduces nitrotyrosine accumulation, preventing tissues from oxidative damage [134]. Furthermore, in insulin-dependent diabetes mellitus (IDDM), high levels of nitric oxide synthase (iNOS) activity result in the accumulation of nitric oxide (NO), which is deleterious to insulin-producing cells. Ebselen antagonizes the effect of NO through scavenging [135]. In a diabetic mouse model, ebselen exhibits beneficial effects on β-cell mass and function [136]. Ebselen also protects from vascular complications in patients with diabetes-mellitus (DM) [137].

Protective role of ebselen against heart disorders

Heart disease (cardiopathy) and cardiovascular diseases are a group of numerous pathological disorders such as heart failure, cardiac arrhythmias; coronary heart disease, hypertension and so forth, in which signaling processes of ROS, RNS and oxidative stress are important features [138, 139]. The novel antioxidant therapies, specifically, use of Gpx1-mimetics holds promise as a targeted antioxidant approach for the treatment of oxidative stress-induced heart diseases. It has been shown that ebselen treatment increases glutathione peroxidase activity in the rat heart [140]. Ebselen is also known to prevent apoptosis induced due to reperfusion of the ischemic heart by reducing the oxidative stress associated with ischemia/reperfusion through GSH preservation [141, 142]. Ebselen also reduces apoptotic cell death during an open-heart surgery by preventing free radical formation [73]. Another report suggests that the ebselen inhibits the blood cell aggregate formation and vessel occlusion in vivo [12]. It also reduces the neutrophil infiltration into the ischemic myocardium [143]. In stroke-prone spontaneously hypertensive rats (SHRsp), ebselen is shown to protect the endothelium and vascular structure during the chronic process of hypertension [144]. In diabetes mellitus, heart associated complications are common. It has been found that in diabetes patients hyperglycemia triggers increased ROS levels can be attenuated by ebselen, leading to protection of endothelial dysfunction [145]. It has been observed that ebselen reduces nitration and restores voltage-gated potassium channel function in small coronary arteries of diabetic rats [137].

Ebselen is very effective drug against chronic iron overload that is a major cause of cardiac failure throughout the world. Ebselen gives the cardioprotective effects from iron overload in the heart of in B6D2F1 mice by inducing increase in GPx activity level [146]. Ebselen also protects from daunorubicin-induced cardiomyopathy. It has been found that subcutaneous administration of ebselen to daunorubicin-treated rats shows significant improvement in serum cardiac indices including creatine kinase isoenzyme

and lactate dehydrogenase as well as serum glutathione (GSH) peroxidase [147]. Ebselen also protects from adriamycin (Adr)-induced cardiotoxicity by attenuating oxidative stress in heart and liver tissue of rats [148]. Altogether, these studies show that ebselen is very effective drug against the cardiopathy.

Ebselen acts as an effective detoxifying agent

Xenobiotic compounds exhibits toxicity through various mechanisms. Xenobiotics that induce the production of ROS are readily attenuated by ebselen due to its anti-oxidative properties. For example, ebselen is highly effective in reducing metal-induced cytotoxicity, such as toxicity caused by cadmium, iron, methylmercury (MeHg), or cisplatin [146, 149–153]. Treatment with ebselen effectively suppresses oxidative stress and protects cells against MeHg-induced cytotoxicity [154]. Ebselen also inhibits MeHg-induced phosphorylation of extracellular-signal-regulated kinase (ERK) in rat primary astrocyte cultures, leading to a block in activation of caspase-3 and protection from apoptosis [153]. Furthermore, ebselen attenuates cadmium-induced testicular damage in mice by reducing oxidative stress parameters [149]. Ebselen is equally effective against toxicity of cisplatin exposure [150, 155]. It has been proposed that the GPx-like activity of ebselen might prevent chronic iron overload in heart tissue [146]. Treatment of B6D2F1 mice with iron plus ebselen decreases both cytotoxic aldehyde and iron concentrations in heart tissue, compared to iron-only treated mice. Similarly, ebselen has been shown to exhibit promising efficacy in attenuating the neurotoxic effects of manganese (Mn) in an in vivo rat model [156].

Ebselen is effective against several toxic compounds and protects against acute lung injuries caused by ozone exposure through modulation of oxidant-related inflammatory processes [29]. Ebselen has also been shown to protect against the effects of diphenyl ditelluride poisoning [157], nitrogen mustards [158] such as mechlorethamine (HN2), and 2-chloroethyl ethyl sulfide (CEES) [159]. It has been demonstrated that pre-treatment of lymphocytes with ebselen reduces DNA damage caused by alloxan [160] or the antibiotic streptozotocin [161]. Additionally, pre-treatment of cells with the ebselen minimizes the extent of DNA damage induced by amsacrine [162] or oxysterol [163]. All together, these studies suggest that ebselen might be a promising detoxifying agent.

Ebselen possesses anti-microbial properties

Ebselen displays antimicrobial activity against several microorganisms. Ebselen has shown anti-malarial activity in vitro against *Plasmodium berghei* and *P. falciparum* [164]. The uptake and incorporation of methionine and adenosine in *P. berghei* was inhibited by ebselen, leading to a reduction in infectivity. Furthermore, ebselen is quite effective against chloroquine-resistant *P. falciparum* strains, inhibiting the development of asexual stages of the parasite and blocking the invasion of erythrocytes by merozoites [164]. Recently, through high-throughput screening (HTS) ebselen has been identified as a potent inhibitor of *P. falciparum* hexokinase (PfHK). The results of biochemical assays suggest that the basis for enzyme inactivation by ebselen is not due to covalent association with the inhibitor, as addition of reducing agent is not sufficient to overcome the inhibition [165]. Ebselen is also shown as a potent inhibitor of the plasma membrane H^+-ATPase Pma1p. Pmap1 is required by a variety of pathogens for their survival. Ebselen blocks the activity of Pma1p of pathogenic *Cryptococcus neoformans* and *Candida albicans* as well as of nonpathogenic yeasts such as *S. cerevisiae* [166, 167]. Recently, ebselen has been shown to inhibit *Pseudomonas aeruginosa* by covalently reacting with cysteine residues in diguanylate cyclases [168]. Interestingly, ebselen also shows potent inhibition of *Mycobacterium tuberculosis*. The antigen 85 (Ag85) complex has emerged as an mycobacterial drug target due to its central role in membrane formation. Mass spectrometry reveals that ebselen binds covalently to a cysteine residue in the Ag85C active site to inhibit of Ag85 complex formation [169]. These studies indicate that ebselen is a potent inhibitor of several pathogenic microorganisms, and suggest that this compound might be useful for the treatment of infectious diseases.

Ebselen as a chemotherapeutic agent

Oxidative stress leads to DNA damage, resulting in the accumulation of genetic instability. Selenium compounds including ebselen show potential chemopreventive activity to protect against carcinogenesis [7, 170]. Ebselen has been shown to reduce the expression of pro-inflammatory cytokines in glioma cells, and to overcome the resistance of these cells to TNF-α-induced apoptosis. The cotreatment of glioblastoma cells with ebselen and TNF-α stimulated glioma cells to undergo apoptosis, suggesting a potential role for ebselen in cancer prevention. Furthermore, ebselen abrogates the invasive potential of glioblastoma cells [171]. Some cancers have been shown to resist TNF-α-induced apoptosis through the stimulation of NF-κB transcriptional activity. Ebselen inhibits TNF-α-mediated NF-κB activity, and sensitizes tumor cells to undergo apoptosis [172]. Additionally, ebselen ameliorates the progression of microvasculopathy and partially restores angiogenesis [173].

Recently, ebselen has been used in several clinical trials. In one of the clinical trials, ebselen has been administered

in the form of granules suspended in water for oral administration in patients. A placebo-controlled clinical trial in 286 SAH patients has revealed that ebselen might be a prophylactic neuroprotective agent [174]. Another clinical trial with 300 acute ischemia patients has also revealed that ebselen treatment has achieved a significantly better therapeutic outcome if patients are administered with ebselen within 24 h of stroke onset [75]. All together, the outcome of these clinical trials is promising and expected that in future ebselen might be used for the treatment of various diseases.

Future directions

The recent growth in the understanding of free radicals biology is leading to a medical revolution that assures a new age of health and disease management [175]. Free radicals and antioxidants are frequently used terms in current discussions of disease mechanisms [176]. ROS are the most common intermediates generated due to metabolic reactions inside the cell or due to interaction with environmental agents. ROS can damage proteins, lipids, DNA, and RNA, and are associated with a number of cell pathologies. Several synthetic antioxidants are recently being reported to be dangerous to human health. Thus, the hunt for effective, non-toxic compounds with antioxidative activity has been intensifying in recent years. As noted above, ebselen is an organoselenium compound that shows considerable antioxidant activity. This compound has the ability to scavenge free radicals, and may be a valuable supplement for the treatment or prevention of many diseases that arise due to dysfunctional cellular redox mechanisms.

The versatile chemical structure of ebselen enables it to interact with a large number of molecules inside the cell, leading to a variety of biological actions. Advances in microarray and mass spectrometry technologies have yielded large output datasets of potential target proteins and genes for therapeutic interventions. Extensive, genome-wide studies on ebselen have not been reported, however, and should be of significant value for the discovery of novel targets for this compound, and target identification from genome-wide information remains a challenge. Additional analytical techniques such as bioinformatics and/or molecular biology tools will be necessary for target validation. This will significantly assist in our understanding of the biological pathways affected by ebselen. Recently, chimeric compounds based on ebselen have been synthesized. For example, selenpezil, which was developed by the fusion of donepezil and ebselen, was seen to exhibit promising activity against Alzheimer's disease [177]. Additionally, a fusion molecule of heptamethine cyanine conjugated to ebselen was used to measure the redox status of cells by fluorescence [178]. Similar approaches that combine ebselen with existing drugs may allow for the discovery of new compounds with enhanced medicinal properties. In pharmacology, it is essential to identify molecular mechanisms of drug action in order to understand adverse side effects. To this end, appropriate efforts should be made so that the pharmacological properties of ebselen can be harnessed, subsequently, diseases associated with oxidative stress may be treated with this interesting compound.

Conclusions

Ebselen is a biologically active compound that affects several cellular processes. Studies with model organisms such as yeast and mice, and in vitro experiments with liposomes, microsomes, and cell lines have shown that the protective effects of ebselen against oxidative stress are due largely to its activity as a GPx mimic. Ebselen has both protective as well as harmful effects on biological systems under different conditions. A number of novel ebselen-based pharmaceutical agents are under intense investigation. Continued research on ebselen and its mechanisms of action are needed to develop potent derivatives of the compound for future pharmacological and therapeutic uses. Innovative approaches employing collaborative research and modern technology in combination with the established health principles will be advantageous in near future for improving health.

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