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Ebselen, a multi-target compound: its effects on biological processes and diseases

Qianqian Lu[1,2], Yi Cai[1,2], Chenyan Xiang[1], Tao Wu[3], Ying Zhao[4], Jun Wang[1], Helen Wang[5] and Lili Zou[1,2]

[1]The Central laboratory, The People's Hospital of China Three Gorges University, 443002 Yichang, Hubei Province, China; [2]The Institute of Infection and Inflammation, Medical College, China Three Gorges University, 443002 Yichang, China; [3]Chongqing Academy of Animal Science, 408599 Chongqing, China; [4]School of Pharmaceutical Sciences, Southwest University, 400715 Chongqing, China and [5]Department of Medical Biochemistry and Microbiology, Uppsala University, 75123 Uppsala, Sweden

Review

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Author for correspondence:
Jun Wang, E-mail: wangjfox@ctgu.edu.cn;
Helen Wang, E-mail: helen.wang@imbim.uu.se;
Lili Zou, E-mail: zoulili@ctgu.edu.cn

Abstract

Ebselen is a well-known synthetic compound mimicking glutathione peroxidase (GPx), which catalyses some vital reactions that protect against oxidative damage. Based on a large number of *in vivo* and *in vitro* studies, various mechanisms have been proposed to explain its actions on multiple targets. It targets thiol-related compounds, including cysteine, glutathione, and thiol proteins (e.g., thioredoxin and thioredoxin reductase). Owing to this, ebselen is a unique multifunctional agent with important effects on inflammation, apoptosis, oxidative stress, cell differentiation, immune regulation and neurodegenerative disease, with anti-microbial, detoxifying and anti-tumour activity. This review summarises the current understanding of the multiple biological processes and molecules targeted by ebselen, and its pharmacological applications.

Introduction

Ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one) is a synthetic seleno-organic compound, exhibiting glutathione peroxidase (GPx)-like activity (Ref. 1), and electrophilic and potential antioxidant properties (Ref. 2). Its general mechanism of action is reaction with essential thiol groups in proteins (Refs 3, 4). The well-known thiol-containing compounds cysteine (Cys/CySS), glutathione (GSH, GSH/GSSG) and proteins thioredoxin (Trx, Trx-(SH)2/Trx-SS), and thioredoxin reductase (TrxR) (Refs 5, 6), are all regulated by ebselen (Ref. 1). Cysteine is one of the least abundant amino acids in proteins and due to its unique nucleophilic thiol group, is able to participate in a broad range of chemical modifications. The nucleophilic thiol group on cysteine is a well-known biological target of ebselen (Ref. 7). GSH is a tripeptide that contains glutamate (Glu), glycine (Gly) and cysteine (Cys). This small peptide is the most abundant intracellular antioxidant (Ref. 8). It is mainly found in the form of reduced GSH (GSH) and oxidised GSH (GSSG), with the vast majority reduced during normal conditions (Ref. 9). It also has auxiliary functions in metabolism and cellular signalling (Ref. 10). As the most abundant store of cellular thiols and the major component maintaining the vital redox equilibrium, intracellular GSH is crucial. Ebselen can bidirectionally regulate GSH metabolism, most commonly removing intracellular GSH by binding its thiols (Refs 4, 11).

Trx is a 12 kDa redox protein, containing five β-strands surrounded by four α helices, a structure known as the Trx fold that is found throughout the oxidoreductase superfamily (Ref. 12). Mammalian TrxRs can reduce both prokaryotic and mammalian Trx proteins, while *Escherichia coli* TrxR has a narrow substrate specificity and catalyses the reduction of only bacterial Trx (Ref. 13). Three isoforms of Trx have been identified in mammalian cells: cytosolic Trx (Trx1), mitochondrial Trx (Trx2) and a Trx variant with increased expression in spermatozoa (SpTrx/Trx3) (Ref. 14). TrxRs are selenium-dependent dimeric flavoproteins 112–130 kDa. The family includes the cytosolic TrxR1 and the mitochondrial TrxR2 (Ref. 15). These reductases are essential in maintaining redox balance in the cytoplasm and mitochondria, respectively (Ref. 16). Both Trx and TrxR are potential therapeutic targets of ebselen. Recent findings show that through inhibiting Trx or TrxR, ebselen increases the production of reactive oxygen species (ROS) in prokaryotic cells (Ref. 17), making TrxR a potential therapeutic target (Refs 18, 19). Thus, the thiol reactivity of ebselen makes it a multi-target molecule that can affect many biological processes.

Ebselen also plays an unprecedented role in regulating cell signal transduction. Ebselen can directly or indirectly regulate the activities of target proteins and exert pharmacological effects on many biological processes. These include anti-inflammatory and (Refs 20–22) pro-apoptotic (Refs 23, 24) activities and effects on oxidative stress (Ref. 25) and cell differentiation (Ref. 26). Ebselen operates in different signalling pathways through various modes of action. It reduces inflammation by affecting the expression of proteins, such as c-Jun N-terminal kinase (Jnk) (Ref. 21), interleukin-2 (IL-2) (Ref. 27) and IL-8 (Ref. 20). Ebselen can positively regulate Jnk (Ref. 24), cytochrome C (Ref. 28), nuclear factor erythroid 2-related factor 2 (Nrf2) (Ref. 25), and negatively regulates caspase 3 (Ref. 29), B cell lymphoma protein-2 (Bcl-2) (Ref. 30) and tumour necrosis factor-α (TNF-α) (Ref. 31). Alterations in

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the expression of these proteins affect apoptosis and oxidative stress. Ebselen influences cell differentiation by regulating smooth-muscle actin (α-SMA) (Ref. 32), phosphoinositide 3-kinase (PI3K), etc. (Ref. 26). Understanding the effects of ebselen on thiol-containing targets will facilitate further studies and the discovery of new pathways that can be used for the development of novel pharmacological strategies.

Based on extensive research, several potential clinical applications of ebselen in the treatment of different diseases have been reported in cell lines studies (Refs 20, 21, 27, 33), animal experiments (Refs 28, 34, 35, 36, 37) and clinical trials (Refs 38, 39, 40, 41). In mammalian cells, ebselen works as a GPx-like compound and peroxiredoxin mimic through Trx and TrxR to scavenge hydrogen peroxide and peroxynitrite (Ref. 17). Recent studies using cell lines have shown that ebselen can be used in the fight against atherosclerosis and chronic kidney disease (Refs 42, 43) as well as cancers, including breast cancer (Ref. 27) and C6 glioma (Ref. 33). Also active in prokaryotic cells, ebselen is a competitive inhibitor of bacterial TrxR and ebselen treatment elevates ROS levels (Ref. 17). Hence, ebselen could help combat infectious organisms. It has inhibitory effects on Gram-positive bacteria (Refs 44, 45), Gram-negative bacteria (Refs 46, 47), and eukaryotic pathogens such as fungi and parasites (Refs 48, 49, 50). Even for viral infections (Ref. 51), particularly COVID-19, ebselen has been shown to have outstanding inhibitory activity. Ebselen's neuroprotective action and ability to counter oxidative stress have been demonstrated in animal experiments using brain disease models, including Alzheimer's disease (AD) (Refs 52, 53), depressive disorder (Ref. 54), brain ischaemia/stroke (Refs 55, 56), cystitis (Ref. 57) and peritonitis (Ref. 47). Clinical trials have confirmed the efficacy of ebselen in acute ischaemic stroke (Ref. 58) and acute middle cerebral artery occlusion (Ref. 59). Including a 320-enrolled-patient-trail revealed that patients who started ebselen within 24 h of stroke onset has a significant improvement than those who started treatment after 24 h, and a 99-recruited-patient-trail determined a corresponding significant reduction in the volume of cerebral infarct of patients who started treatment within 6 h of onset. Meanwhile, following ongoing clinical trials showed that ebselen is also a promising compound in treating diabetes mellitus (ClinicalTrial.gov NCT00762671), Meniere's disease (ClinicalTrial.gov NCT03325790, NCT02603081), bipolar disorder (ClinicalTrial.gov NCT03013400), hearing loss (ClinicalTrial.gov NCT01452607), ototoxicity (ClinicalTrial.gov NCT02819856), temporary auditory threshold shift (Clinical Trial.gov NCT01444846) and other conditions. Thus far, a large number of studies have demonstrated that ebselen has multiple pharmacological effects. In short, though ebselen has not yet been approved as a drug for any specific disease, its efficacy and tolerance in humans has shown that ebselen can be a promising therapeutic compound.

The aim of this review is to summarise our current understanding of the multiple molecular targets of ebselen and the biological processes and its effect on diseases, in order to help the future exploration of novel targets and develop new pharmacological uses for this compound (Fig. 1).

Targeting thiol-containing compounds

Ebselen acts mainly by targeting intracellular thiol-containing macromolecules, particularly cysteine, GSH and thiol proteins (e.g., Trx and TrxR) which are listed in Table 1.

Targeting cysteine

Cysteine is one of the most inherently reactive amino acids, and has a variety of important biochemical functions such as catalysis and redox regulation (Ref. 60). The unique chemical properties of the thiol group in cysteine are involved in different biological transformations and functional sites, such as disulphide (S–S) bond (Refs 61, 62). As a common target for covalent inhibitors, the highly nucleophilic cysteine thiol provides an ideal anchor for electrophilic molecules (Ref. 63). Under various conditions, ebselen can react with a large number of cysteine thiols (Refs 2, 64, 65). It inhibits the activation of superoxide dismutase-1 (SOD1), it is known to cause amyotrophic lateral sclerosis (ALS); New Delhi metallo-b-lactamase (NDM-1), an enzyme resistant to a wide range of β-lactams and quiescin sulfhydryl oxidase 1 (QSOX1), a highly conserved disulphide bond generating enzyme that is overexpressed in diverse tumour types by reacting with cysteine residues and forming a selenium-sulphide (Se-S) bond or covalently modifying cysteine residues (Ref. 66), including Cys-111 (Refs 67, 68), Cys-221 (Refs 69, 70), and Cys-165 and Cys-237 (Ref. 71). Joice *et al.* have reported that ebselen can modify Cys-327 in *Trypanosoma brucei* hexokinase via thiol oxidation (Ref. 72). Leroux *et al.* also have demonstrated that ebselen can mediate by disrupting Insulin-Degrading Enzyme (IDE) dimerisation, and reduce the HD exchange of Cys-(181–198) (Ref. 73). The most striking finding is that ebselen exhibits promising antiviral activity against COVID-19: it inhibits the enzymatic activity of M^{pro} through covalent bonding to the catalytic cysteine (Refs 74, 75, 76). M^{pro} is a key enzyme of COVID-19, that plays a pivotal role in mediating viral replication and transcription, making it an attractive target (Refs 77, 78). Besides, the papain-like protease (PL^{pro}) from the human coronavirus is a protease that plays a critical role in virus replication, Tomczak *et al.* have reported that ebselen and its analogues are able to modify Cys-111 of PL^{pro} in the active site (Ref. 79). In summary, ebselen reacts with the thiol group in cysteine and the reactivity between ebselen and cysteine makes it an effective modulator of this essential amino acid.

Targeting GSH

GSH is a tripeptide that contains a thiol group and is the most abundant low molecular-weight thiol compound in mammalian cells. It is essential as an antioxidant and in maintaining homeostasis (Refs 80, 81). Due to its important role in the cell, many studies have indicated that GSH can be a therapeutic target (Refs 29, 48). When ebselen is present, it can react with the SH group on GSH and affect GSH levels (Ref. 82) (Fig. 2). As a target of ebselen, GSH plays different roles in various diseases. The regulation of GSH by ebselen in different diseases is bidirectional. The main effect of ebselen on GSH is to regulate its levels to maintain microenvironmental homeostasis. For example, ebselen increases the steady-state level of GSH against (arsenite)-induced nephrotoxicity in female rats (Ref. 83). Ebselen protects against manganese toxicity to male reproductive organs and male fertility was proven to be achieved by increasing the GSH activity (Ref. 84). The myocardial GSH level is preserved by oral ebselen in an ischaemia-reperfusion model (Ref. 85). Ebselen can also directly remove intracellular GSH by binding to its SH group, leading to apoptosis (Refs 4, 33). Ebselen and silver ions react with the SH group of GSH in gram-negative bacteria and rapidly deplete GSH thiols (Ref. 47). GSH itself is a redox buffer but also supplies electrons to glutathione reductases and other proteins. When oxidised, GSSG can be reduced by glutaredoxin, which utilises NADPH as its ultimate electron source. Thus, ebselen can regulate the amount of GSH in the cell and influence a broad range of biological processes.

Targeting other thiol proteins

Ebselen is an effective inhibitor of TrxR in both tumour cells and bacteria. TrxR is now being studied clinically as a molecular marker for some tumours. Ebselen blocks electron transport in

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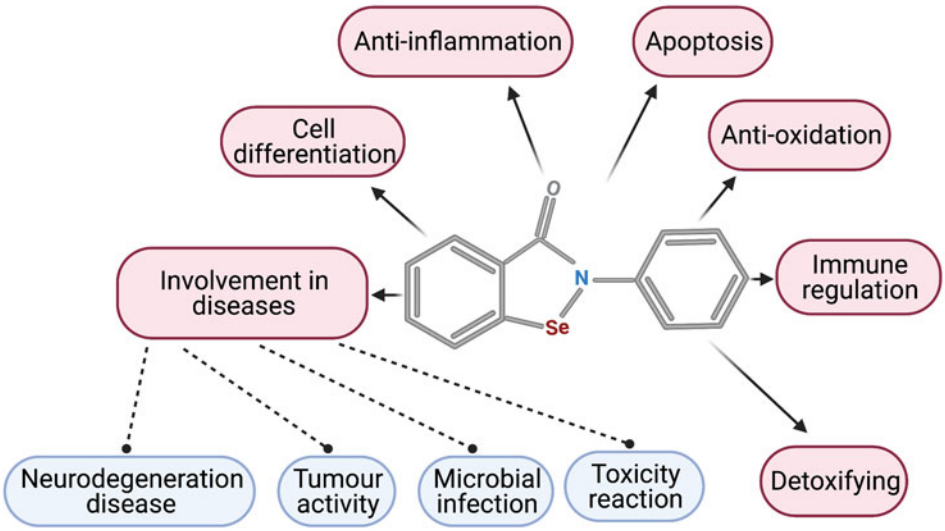

Fig. 1. Bioactivity and chemical structure of ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one).

tumour cell TrxR (Ref. 86). Ebselen inhibits TrxR activity by binding the cysteine residue at the active site (Ref. 87). Bacteria are classified as gram-positive or gram-negative bacteria; the former lacks GSH and glutaredoxin, while the Trx system is essential for bacterial DNA synthesis. The bacterial Trx system is particularly sensitive to ebselen (Ref. 88). *Escherichia coli* TrxR, similar to other low molecular weight bacterial TrxRs, possesses only the FAD and NADPH binding domains and lack the interface domains found in mammalian TrxRs. Mammalian TrxRs are larger and contain an N-terminal active site motif, CVNVGC, and C-terminal amino-acid extension with a selenocysteine (U) in a GCUC active site motif. For mammalian TrxR, ebselen reacts with the selenocysteine in the C-terminal GCUG- active motif. And the adjacent cysteine can resolve the diselenide bonds formed between ebselen and Sec, indicating that mammalian TrxR cannot be inhibited by ebselen easily (Ref. 82). Ebselen is a competitive inhibitor of *E. coli* TrxR with a K_i of 0.52 ± 0.13 μM, reacting with the active site dithiol (Ref. 89). In *vitro*, ebselen was found to inhibit *E. coli* TrxR through forming a difficult-to-reduce selenosulphide bond (Se-S). And ebselen blocked the electron flow from TrxR to Trx, then to its substrates, such as RNR and Msr (Ref. 88). Therefore, ebselen is a highly effective antibiotic that can act on the Trx system and against different kinds of bacteria (Refs 44, 45). A recent study sheds light on the antioxidant mechanism of ebselen, revealing that form ebselen selenol and diselenide by reacting with Trx and TrxR rather than with GSH (Ref. 90). Multiple studies suggest that ebselen inhibits TrxR in bacteria, such as *Staphylococcus aureus* (Ref. 91), *Aspergillus fumigatus* (Ref. 16), *Bacillus anthracis* (Ref. 19) and *Deinococcus radiodurans* (Ref. 92). Additionally, ebselen is more effective in combination with other compounds. Silver can act synergistically with ebselen against multidrug-resistant gram-negative bacteria by directly reacting with the SH group in TrxR (Ref. 47). Ebselen also can cooperate with PX-12, targeting microbial Trx system (Ref. 18). These results demonstrate that ebselen targets the Trx system, exhibiting unprecedented antimicrobial activity, and provide a proof of concept of ebselen's efficacy against a variety of bacterial infections.

Involvement In biological processes

Ebselen is a polytropic molecule that interacts with many thiol-containing targets inside the cell, directly or indirectly regulating the expression of proteins or the transcription of genes. Due to its interaction with a broad range of biomolecules, ebselen is likely to influence several pathophysiologic processes.

Anti-inflammatory

Inflammation Is caused by acute trauma and the invasion of the host by different pathogens (Ref. 93). The classic inflammation-related pathways are the nuclear factor kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) pathways, which have recently been shown to also regulate different aspects of innate or adaptive immune responses (Refs 94, 95). *In vivo* and *in vitro* studies have demonstrated that ebselen exhibits anti-inflammatory effects by regulating the expression of inflammatory cytokines. It has been suggested that selenium modulates inflammation by suppressing the NF-κB and MAPK signalling pathways in RAW264.7 macrophages. Ebselen also downregulates Erk, Jnk and p38 phosphorylation levels through MAPK signalling pathways in *Staphylococcus aureus*-induced mastitis (Ref. 21). In endothelial cells, results suggest that ebselen affects the TRAF2-ASK1-SEK1 signalling pathway to reduce inflammation especially in atherosclerosis (Ref. 42). In human GC cell lines, ebselen has been shown to decrease the generation of IL-8 by inhibiting p38 MAPK phosphorylation. *Helicobacter pylori* lipopolysaccharide suppresses (GPx2/4) the activation of ROS, which also mediates IL-8 expression, and ebselen reverses these effects (Ref. 20). By regulating gene and protein expression, and modulating the responses of pro- and anti-inflammatory cytokines, the combination of ebselen and γ radiation is able to decrease inflammation by suppressing TNF-α, IL-2, interferon (INF-γ), TGF-β expression levels and significantly increase IL-10 levels in breast cancer cells (Ref. 27). In animal models, ebselen treatment significantly reduces the levels of Toll-like receptor 4 (TLR4) and p-p38 MAPK, highlighting the role of the TLR4-p38 MAPK signalling pathway in anti-inflammatory processes in traumatic brain injury (Ref. 30). Ebselen is also reported to inhibit the expression of TNF-α and attenuate bronchiolar inflammation in a sephadex-induced lung inflammation rat model (Ref. 96). Taken together, the results of these studies suggest that ebselen exerts anti-inflammatory effects on various stimuli and targets in different biological pathways (Fig. 3).

Apoptosis

Apoptosis is the spontaneous and orderly death of cells that is controlled by genes and plays an important role in maintaining normal homeostasis and the pathophysiological processes of various diseases. Ebselen is confirmed to prevent apoptosis caused by exposure to harmful environments or injuries. Ebselen attenuates oxidative

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Table 1. List of thiol compounds affected by ebselen

No.	Thiol compounds	Action of ebselen	References
1	New Delhi metallo-b-lactamase (NDM-1) ↓	Forms an S-Se bond with Cys-221 residue at the active site	(Refs 69, 70)
2	SARS-CoV-2 M^{pro} ↓	Binds covalently to cysteine via sulfhydryl groups	(Refs 74, 76)
3	Superoxide dismutase-1 (SOD1) ↓	Forms a selenylsulphide with Cys-111	(Refs 67, 68)
4	Diguanylate cyclases (DGC) ↓	Covalently modifies cysteine residues	(Ref. 66)
5	Indoleamine 2,3-dioxygenase (IDO) ↓	Reacts with the enzyme's cysteine residues	(Ref. 65)
6	Quiescin sulfhydryl oxidase 1 (QSOX1) ↓	Binds covalently to Cys-165 and Cys-237	(Ref. 71)
7	*T. brucei* hexokinase 1 (TbHK1) ↓	Modifies of Cys-327 via thiol oxidation	(Ref. 72)
8	Glutamate dehydrogenase (GDH) ↓	Interacts with cysteine residues	(Ref. 64)
9	Insulin-degrading enzyme (IDE) ↓	Reduces the HD exchange of Cys-(181–198)	(Ref. 73)
10	Papani-like protease (PL^{pro}) ↓	Modifies Cys-111 in the active site	(Ref. 79)
11	Glutathione (GSH) ↓	Affects glutathione metabolism	(Ref. 48)
		Consumes GSH by binding to it	(Ref. 33)
		Binds covalently to GSH	(Ref. 4)
		Reacts with the SH group in GSH	(Ref. 47)
		Reduces the depletion of GSH	(Ref. 141)
12	Glutathione (GSH) ↑	Increases the steady-state GSH level or the rate of synthesis	(Ref. 83)
		Increases GSH content	(Ref. 100)
		Increases the activity of GSH	(Ref. 84)
		Improves the synthesis of GSH	(Ref. 85)
13	Thioredoxin reductase (TrxR) ↓	Blocks the electron transfer through the Trx system	(Refs 18, 88)
		Reacts with TrxR to form a Se-Se bond	(Ref. 91)
		Reacts with an SH group in TrxR	(Ref. 47)
		TrxR acting with ebselen diselenide	(Ref. 90)
		Forms a covalent bond with a catalytic cysteine in TrxR, Cys-148	(Ref. 16)
		Blocks TrxR function and thus the redox system	(Ref. 92)
		Cross-link through the cysteine residue at the active site of TrxR	(Ref. 87)

stress-induced neuronal cell apoptosis by inhibiting the JNK and activator protein-1 (AP-1) signalling pathways in PC12 cells (Ref. 24). In these cells, apoptosis is also induced by NO, and ebselen can stimulate the activation of p44/42 MAPK, which may antagonise the activity of p38 MAPK and JNK, leading to a mitochondrial permeability transition and release of cytochrome C (Ref. 97). Similarly, in H_2O_2-induced endothelial cell apoptosis, ebselen decreases the expression of p38 MAP kinase, caspase-3 activation and cytochrome C release leading to reduced apoptosis (Ref. 98). Through attenuating ischaemia-reperfusion injury and pancreatic β-cell apoptosis, ebselen inhibits the expression of IL-1β and TNF-α (Ref. 31). Mitochondria are also intracellular apoptosis targets of ebselen (Ref. 99). Ebselen can downregulate the expression of cytochrome C to rescue mitochondrial apoptosis in a liver mitochondria damage model induced by Fe^{2+}/citrate (Ref. 28). Ebselen can also increase the Bcl-2:Bax ratio, inhibit the release of cytochrome C and Smac, and increase the activation of caspase-3, suppressing the mitochondrial apoptosis pathway after acute spinal cord injury (Ref. 100). These studies all show ebselen's effects on apoptotic pathways under different experimental conditions (Fig. 4).

Oxidative stress

Oxidative stress is an imbalance between ROS and their elimination. It is triggered by excessive production of highly reactive molecules (ROS and reactive nitrogen species) when the body is exposed to various harmful stimuli (Ref. 101). Ebselen is a potent antioxidant and is very effective in maintaining redox balance in the body. CXCR4 has been shown to activate Akt through ROS-dependent pathways and CD40 activates JNK, p38 and Akt via signalling pathways that are largely ROS-dependent. But ebselen can deplete intracellular ROS, reducing oxidative stress and modulating B cell activation (Ref. 102), and attenuates oxidative stress by inhibiting the JNK and AP-1 pathways (Ref. 103). Cisplatin-induced ROS generation is reduced by ebselen through the Nrf2-ARE pathway in auditory cells (Ref. 25). Ebselen protects NIH/3T3 cells against oxidative stress by regulating the expression of Bcl-2 and p53 (Ref. 104). These results demonstrated that ebselen regulates oxidative stress through various signalling pathways and is a promising antioxidant (Fig. 5).

Cell differentiation

Cell differentiation refers to the process whereby cells from the same source gradually produce cell groups with different morphologies and functions. The essence of cell differentiation is the selective expression of a subset of the genes present in the genome in time and space. Some studies have shown that ebselen can regulate the differentiation of various cell types. Stimulation with ebselen may activate the intracellular tyrosine kinase-MEK1/2-ERK1/2 signalling pathway in PC12 cells and lead to neuronal differentiation (Ref. 105). Ebselen also promotes osteogenic differentiation,

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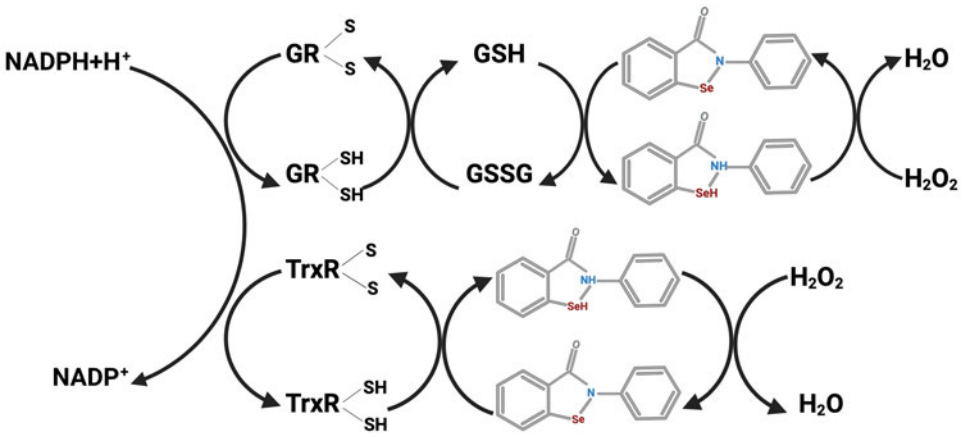

Fig. 2. Mechanisms of ebselen action on TrxR and GSH.

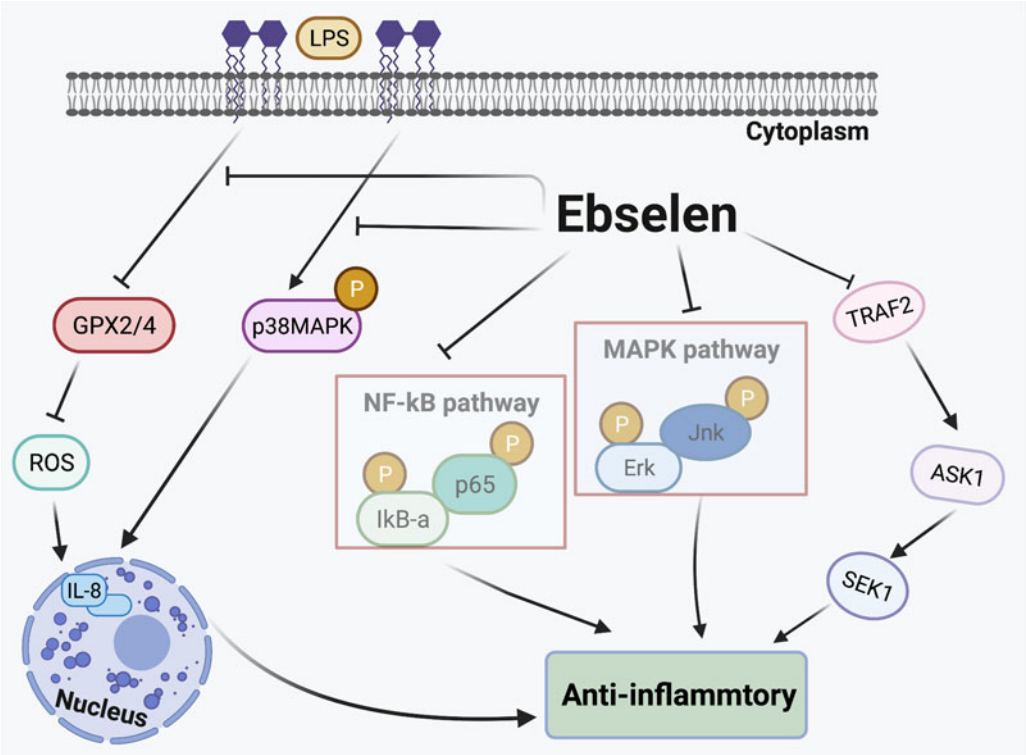

Fig. 3. Anti-inflammatory effects of ebselen. Selenium inhibits the MAPK and NF-κB signalling pathways. Ebselen suppresses the TRAF2-ASK1-SEK1 signalling pathway, inhibiting inflammation. *Helicobacter pylori* lipopolysaccharide (LPS) suppresses glutathione peroxidase (GPX2/4) activation of reactive oxygen species (ROS), which also mediates IL-8 expression; ebselen reverses these effects. TRAF2, TNF receptor-associated factor 2; ASK1, apoptosis signal-regulated kinase 1; SEK1, phosphorylation of stress-activated protein kinase ERK kinase 1; p38 MAPK, p38 mitogen-activated protein kinase; LPS, lipopolysaccharide; GPX, glutathione peroxidase; IL-8, interleukin 8.

in part by modulating the PI3K-Akt signalling pathway (Ref. 26). Treatment with ebselen in the early stages of osteoclast differentiation negatively controls the formation and survival of osteoclasts by targeting the Akt/NF-κB pathway and preventing trabecular bone matrix degradation as well as osteoclast formation in bone tissues (Ref. 106). As an inhibitor, ebselen suppresses TGF-β induced α-SMA mRNA and protein expression involved in myofibroblast differentiation in nasal polyps (Ref. 32). In short, ebselen influences cell differentiation by regulating the expression of various proteins (Fig. 6).

Immune regulation

In immune regulation, ebselen acts as either a cytokine inducer or immune stimulant (Refs 107, 108). Under certain conditions, ebselen may have anti-immunosenescent potential in clonal T cells *in vitro* and *ex vivo* in polyclonal culture models (Ref. 109). There are several reports that suggest that many inflammatory factors are activated and released when cells are treated with ebselen, including IL-2, IL-8, and IL-10 (Refs 20, 27, 30). Additionally, ebselen mediates the expression of intercellular adhesion molecule-1, TNF-α, INF-γ and TGF-β, which affect the adhesion and migration of immune cells and promote their anti-inflammatory properties (Refs 27, 43). All of these studies demonstrate that ebselen affects immune regulation and suggest it has anti-inflammatory activity.

Involvement in disease

Due to excessive production of free radicals leading to oxidative stress, the biological processes result in age-related diseases such as AD, Parkinson disease (PD) and others. As an antioxidant,

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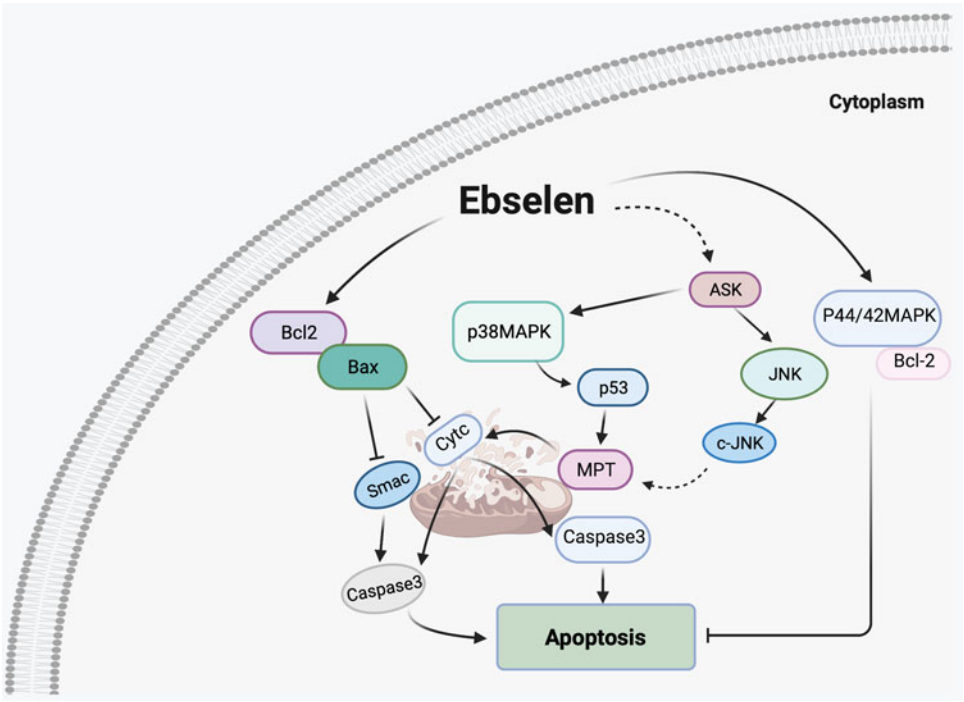

Fig. 4. The effects of ebselen on apoptosis. Ebselen actives p44/42MAPK and Bcl-2 to inhibit cell apoptosis, possibly by antagonizing the ASK1-p38MAPK-p53-MPT-CytC-caspase3 and ASK1-JNK-c-Jnk-MPT-CytC-Caspase3 signalling pathways. Ebselen increases the Bcl-2:Bax ratio, releasing cytochrome C, Smac and activating caspase3 to inhibit mitochondrial apoptosis. p44/42MAPK, p44/42 mitogen-activated protein kinase; MPT, mitochondrial permeability transition; CytC, cytochrome C; JNK: c-Jun N-terminal kinase; AP-1: activator protein-1; IL-1β: interleukin 1β.

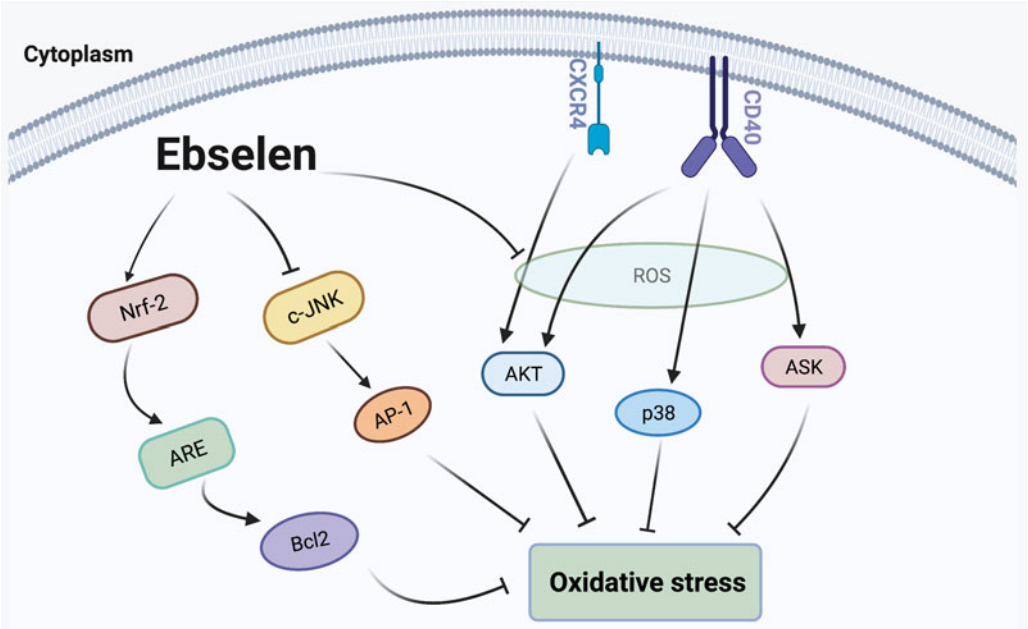

Fig. 5. The effects of ebselen on oxidative stress. CXCR4 actives Akt through ROS-dependent pathways, CD40 activates JNK, p38, and Akt via signalling pathways that are largely ROS-dependent, and ebselen inhibits the ROS in these signalling pathways to suppress oxidative stress. Ebselen inhibits the c-JNK-AP-1 signalling pathways and p53 protein to attenuate oxidative stress. Ebselen activates the Nrf2-ARE signalling pathway and upregulates Bcl-2 protein expression, countering oxidative stress. CXCR4, chemokine receptor 4; Nrf2, nuclear factor erythroid 2-related factor 2; ARE, antioxidant response element.

ebselen supports the free radical scavenging potential of the cell and has important pharmacological effects. Ebselen is also reported to be promising as an anti-microbial compound, and affects immune regulation, detoxification and oncotherapy.

Neurodegenerative disease

Neurodegenerative disease is caused by the loss of neurons or their myelin sheaths, such as in AD, PD, ALS, ischaemic stroke, etc. As one of the most common chronic neurodegenerative diseases, AD is the single biggest cause of dementia (Ref. 110). Recent studies indicate that ebselen may be an excellent candidate AD treatment (Ref. 111). Ebselen can work effectively against oxidative stress through different actions such as increasing the activity of GPx and SOD, as well as reducing the levels of β-amyloid in an AD model (Refs 52, 53, 112). The neuroprotective effect of ebselen is also related to iron-induced τ phosphorylation that is reduced by inhibiting DMT1 (Ref. 113). PD is the second most common neurodegenerative disorder after AD, affecting millions of people worldwide (Ref. 114). Recent findings revealed that

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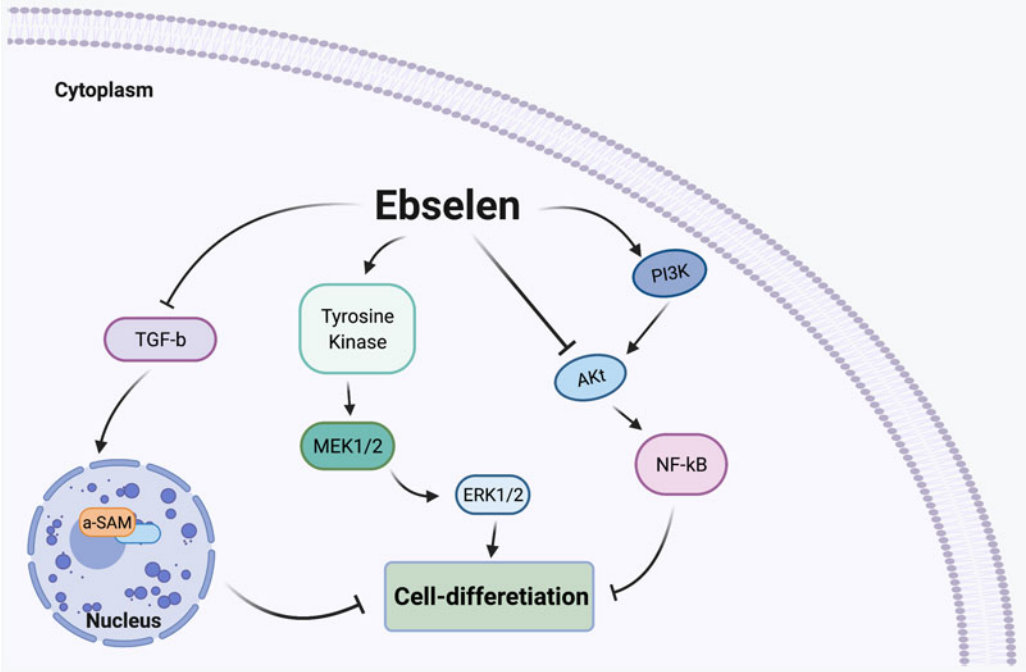

Fig. 6. The effects of ebselen on cell differentiation. Ebselen stimulates the intracellular tyrosine kinase MEK1/2-ERK1/2 and PI3 K-Akt signalling pathways to induce cell differentiation. Ebselen inhibits the Akt-NF-κB and TGF-β-α-SMA signalling pathways against cell differentiation. MEK1/2, MAPK kinase; ERK, extracellular-signal-regulated kinase; PI3K, phosphatidylinositol 3-kinase; Akt, AKR thymoma protein kinase; TGF-β, transforming growth factor β; α-SMA, smooth-muscle α actin.

one common cause of PD is mutations in CHCHD2 and LRRK2, and that ebselen may be a useful neuroprotective compound for carriers of CHCHD2 (Ref. 115) and LRRK2 mutations (Ref. 116). Ebselen also has a protective effect in ALS, as the compound can react with SOD1 mutant variants, including those with unaltered enzymatic activity, that are known to cause ALS (Refs 67, 117).

Ischaemic stroke is the most common type of stroke, in which blood vessels in the neck or brain are blocked (Ref. 118). Ebselen has shown some clinical efficacy in ischaemic stroke by blocking the production of superoxide radicals and inhibiting inducible NO synthase (Ref. 119). *In vivo* experiments suggest that ebselen alleviates stroke through its antioxidant activity (Ref. 120). Most published results indicate that ebselen works in the treatment of neurodegenerative diseases by reducing neuronal damage (Refs 121, 122, 123, 124, 125) and decreasing the activity of both malonaldehyde and NO (Ref. 55). Collectively, these results suggest that the neuroprotective activity of ebselen is unprecedented.

Microbial infection

The inhibition of several microorganisms by ebselen shows its strong anti-microbial activity. For instance, ebselen inhibits biofilm formation and disrupts mature biofilms of *vancomycin-resistant enterococci* (Ref. 126). Ebselen inhibits *Mycobacterium tuberculosis* antigen 85 by covalently binding to a cysteine residue in the active site (Refs 127, 128). In recent years, our laboratory has been committed to studies of ebselen's action against Gram-positive and Gram-negative bacteria, and we have been looking for compounds that can act synergistically with ebselen. We have found that ebselen is an effective topical antibacterial agent in an animal model of multidrug-resistance (MDR) *S. aureus* LT-1 skin infection (Ref. 91). A combination of silver ions and ebselen might be developed for novel treatment against MDR Gram-negative bacterial infections (Ref. 47). These treatments have been shown to work against MDR *Acinetobacter baumannii* urinary tract infections (Ref. 129) and in an MDR uropathogenic *E. coli* (UPEC) BC1-induced mouse cystitis model (Ref. 57). All the above-mentioned results are recent findings from our laboratory and we are still investigating the specific mechanisms of ebselen's anti-bacterial activity. Other studies have shown that ebselen does not affect mitochondrial function and that it inhibits translation, including the synthesis of toxic proteins (Ref. 45), in methicillin-resistant *S. aureus* (Ref. 44). Additionally, the inhibitory effects of ebselen on *T. brucei hexokinase 1* (Ref. 72) and *P. falciparum* growth (Ref. 130) further support the notion that ebselen is effective against microorganisms. Together, these studies reveal that ebselen is a promising agent for use against pathogenic bacteria and that it can be used to prevent the occurrence of infectious diseases.

Anti-tumour activity

A tumour is produced by the local proliferation of tissue cells in the body under the action of various tumorigenic factors. Many studies indicate that ebselen acts as an anti-tumour agent, targeting various tumours. The combination of ebselen and allopurinol was chemoprotective and increased the anti-tumour activity of cisplatin in rat breast and ovarian cancer models (Ref. 36). Ebselen and γ radiation induce apoptosis and inhibit cancer progression in breast cancer cells (Ref. 27). Ebselen also activates the apoptosis pathway and induces cell death in AR42J tumour cells (Ref. 131). Ebselen inhibits pancreatic and renal cancer cell lines tumour growth *in vitro* and their invasion of matrigel (Ref. 71). Additionally, ebselen ameliorates ovarian damage by reducing oxidative stress caused by cisplatin (Ref. 37). The administration of ebselen can induce apoptosis in multiple myeloma (MM) cell lines by enhancing the production of endogenous ROS (Ref. 132). Ebselen inhibits the activity of human methionine aminopeptidase 2 in solid tumour cancers (Ref. 133). Ebselen was shown to block ADAM9 (a disintegrin and metalloprotease, pro-cancer protein) through the inhibition of ROS production in human prostate cancer cells (Ref. 134) and suppresses cancer biomarker α-methylacyl coenzyme A racemase levels (Ref. 135). The dose-dependent cell death of multiple tumour

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cell lines after treatment with ebselen results from its inhibition of histone deacetylases (Ref. 136). Thus, the anti-tumour activity of ebselen is outstanding.

Toxicity reaction

Recent studies suggest that ebselen can reduce the toxicity of some compounds or metals. For example, ebselen is highly effective in reducing the toxicity of the metals cadmium, manganese, iron and mercury (Refs 137, 138, 139, 140). Ebselen can also reverse cisplatin-induced nephrotoxicity by rescuing GSH depletion in the kidney in a rat model (Ref. 141). The level of GSH has been shown to rise after ebselen treatment in acute spinal cord injury (Ref. 100). Detoxification of 3-nitropropionic acid-induced toxicity by ebselen in rats has been reported (Ref. 142). Ebselen decreased the toxicity of mechlorethamine in A-431 cells by inhibiting apoptosis (Ref. 143). Hyperphosphorylation of cytoskeletal proteins induced by the neurotoxic agent diphenyl ditelluride in the cerebral cortex of young rats is prevented by ebselen treatment (Ref. 144). In sum, ebselen is a powerful detoxifying compound.

Future directions and conclusions

With the worldwide outbreak of COVID-19, there is an urgent need to find a drug to fight the virus. A recent study reported that ebselen was the strongest inhibitor of viral M^{pro} activity among the compounds tested (Ref. 74). Ebselen has now entered clinical trials in COVID-19 patients; with proven efficacy, it could save drug developers much time in dealing with this sudden public health issue. Sometimes developing new targets for old compounds can be a good research direction.

Ebselen can affect a variety of biological processes, and has been shown to be effective against numerous pathological conditions in clinical trials, animal models and *in vitro* experiments. Some studies have reported that combining ebselen with other existing compounds, such as silver nanoparticles or curcumin, may lead to enhanced medicinal properties (Refs 91, 145). Thus, the exploration of ebselen combinations in disease treatment is promising.

Although ebselen is recognised as a compound with low toxicity *in vivo*, at high doses, it can cause toxic effects, which vary a lot according to the species, exposure time and route of administration. In acute exposure, the toxicity of ebselen varies depending on the route of administration, age and species in rodents (Ref. 146). *In vivo* chronic toxicity data with ebselen show that chronic subcutaneous administration of the compound (10 mg/kg, for 21 days) to suckling rats culminated with lipid peroxidation and non-protein thiol depletion in the liver (Ref. 147). To date, the toxicological effects of acute and chronic exposures to ebselen in mammalian models have not been widely reported in the literature, and the specific mechanism remains to be fully elucidated.

Recently, there have been numerous reports of ebselen derivatives, such as the organoselenium compound (*N*-allyl-1,2-benzisoselenazol-3(2H)-one (N-allyl-BS)) and novel selenocyanates, having anti-tumour activity (Refs 148, 149). Seeking novel ebselen derivatives for the treatment of disease and to determine the molecular mechanism of their action is a promising line of research. These new ebselen derivatives or compounds acting synergistically with ebselen may open new alternatives for the treatment of different diseases. Further studies of ebselen will continue to provide new ideas for disease prevention and treatment, with good prospects for broad application.

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