Peer-reviewed version available at *Antioxidants* **2020**, *9*, 727; doi:10.3390/antiox9080727

Review

Pursuing the Elixir of Life: *In vivo* antioxidative effect of manganosalen complexes, a review

Lara Rouco [1], Ana M. González-Noya [2], Rosa Pedrido [2], and Marcelino Maneiro [1,*]

[1] Departamento de Química Inorgánica, Facultade de Ciencias, Universidade de Santiago de Compostela, 27002 Lugo, Spain; E-mails: lara.rouco.mendez@usc.es (L.R.); marcelino.maneiro@usc.es (M.M.)

[2] Departamento de Química Inorgánica, Facultade de Química, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain; E-Mails: ana.gonzalez.noya@usc.es (A. M. G.); rosa.pedrido@usc.es (R. P.)

* Correspondence: marcelino.maneiro@usc.es; Tel.: +34-982-824-106

Abstract: Manganosalen complexes are coordination compounds that possess a chelating salen-type ligand, a class of bis-Schiff bases obtained by condensation of salicylaldehyde and a diamine. They may act as catalytic antioxidants mimicking both the structure and the reactivity of the native antioxidant enzymes active site. Thus, manganosalen complexes have shown to exhibit superoxide dismutase, catalase, and glutathione peroxidase activities, and they could potentially facilitate the scavenging of excess ROS, thereby restoring the redox balance in the damaged cells and organs. Initial catalytic studies compared the potency of these compounds as antioxidants in terms of rate constants of the chemical reactivity against ROS, giving catalytic values approaching and even exceeding that of the native antioxidative enzymes. Although most of these catalytic studies lack of biological relevance, subsequent *in vitro* studies have confirmed the efficiency of many manganosalen complexes in oxidative stress models. These synthetic catalytic scavengers, cheaper than natural antioxidants, have accordingly attracted intensive attention for the therapy of ROS-mediated injuries. The aim of this review is to focus on *in vivo* studies performed on manganosalen complexes and their activity on the treatment of several pathological disorders associated with oxidative damage. This disorders, ranging from the prevention of fetal malformations to the extension of lifespan, include neurodegenerative, inflammatory and cardiovascular diseases, tissue injury, and other damages related to liver, kidney or lungs.

Keywords: ROS; oxidative stress; catalytic antioxidants; superoxide dismutase; catalase; peroxidase; manganese; salen-type ligands; animal studies

1. Introduction

In 2000 a research paper by Melov *et al.* [1] reported the extension of lifespan of nematode worms (*Caenorhabditis elegans*) by treatment with different manganosalen complexes which act as synthetic scavenger compounds. This study was planned to test the theory that reactive oxidative species cause aging. In announcing this research, Melov stated that "the results are the first real indication we have that aging is a condition that can be treated through appropriate drug therapy" [2]. The research paper and this statement attracted extensive coverage in the communications media, where these findings were presented as a sort of search for the Elixir of Life. The published results indicate that the two tested compounds (named as EUK-8 and EUK-134) increased the mean lifespan of the worms by 44 percent over the control group, and treatment of prematurely aging worms resulted in normalization of their lifespan, which means a 67 percent increase. However, the chosen nematode

worm is a species that has 959 cells in its body whereas humans have 100 trillion, constituting one of the weaknesses of animal models for translational research [3]. To investigate the protective activity and the increase of longevity of the manganosalen catalytic antioxidants in a mammalian animal model, Melov *et al.* used mice lacking SOD2, the mitochondrial form of superoxide dismutase [4]. The genetically engineered SOD2 nullizygous mice lack the oxygen-scavenging enzyme that helps protect mitochondria from free radicals, and they usually die within the first week of life by suffering different pathologies. When the mice were injected with the manganosalen complexes (EUK-8, EUK-134 and the compound named as EUK-189), they lived three times longer. In this study the manganosalen complexes also rescued mice from oxidative neurodegenerative process, and the results suggest that this class of synthetic catalytic antioxidants can permeate the brain, gain access to the mitochondria, and attenuate the mitochondrial damage attributable to oxidative stress.

Experimental approaches have yielded contradictory evidences about the therapeutic attenuation of aging using these synthetic compounds [5], which will be discussed further below. However, a significant number of *in vivo* experimental studies have shown that these complexes exhibit remarkable efficacy in several animal models suffering from oxidative stress injuries [6-10]. The most common animal model is that of rats and different types of mice, but in addition to these and the mentioned nematodes, studies were also carried out on pigs, sheep, fish or using the vinegar fly. Additionally there are also studies of protective effect against induced oxidative stress in other models than animals, like *E. Coli* [11] and also in humans with an evaluation of the beneficial effects over UVA-exposed skin *in vivo* [12] .

After more than two decades of *in vivo* studies using manganosalen complexes, the focus of this review is on the evaluation of their effectiveness on the treatment of several pathological disorders associated with oxidative damage: aging, neurodegenerative diseases and mental disorders, inflammatory, cardiovascular or liver diseases, skin damage, fetal malformations and other damages related to kidney or lungs. Moreover, this review collects recent advances in the state of knowledge on the molecular mechanisms for the antioxidant activity of these compounds [13-23].

2. Manganese superoxide dismutases, peroxidases and catalases

Reactive oxygen species (ROS) are partially reduced metabolites of molecular oxygen formed in biological systems as a result of a normal cellular metabolism, primarily in the mitochondria [24]. Exogenous sources such as pollutants [25], smoke [26], radiation [27] or heavy metals [28] also may increase the ROS levels. Superoxide radical anion ($^{\bullet}O_2^-$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$), lipid hydroperoxide (LOOH), lipid radical ($L^{\bullet}$), peroxyl radical ($^{\bullet}ROO$), peroxynitrate ($ONOO^-$), and other radicals which can be produced through a sequence of reactions, constitute the designated ROS. Free radicals are generated when oxygen interacts with certain molecules leading to highly reactive species with one or more unpaired electron(s). ROS may play a role in signaling functions, consequently activating protective and adaptative programs [29-30]. Nevertheless, excessive ROS levels cause oxidation of organic molecules with alteration of their structure and biological functions, the activation of phagocytes, the release of cytokines or the activation of oncogenes [31-35]. These processes lead to different pathologies in humans, such as carcinogenesis [36-37], inflammatory illnesses [38], diabetes type II [39], cellular senescence [40] and different neurodegenerative diseases [41-42].

ROS levels are regulated in the living systems by the antioxidant enzymes, which basically include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), peroxiredoxins (PRDXs), thioredoxin 2 (TRX2) or cytochrome c oxidase (complex IV). Other non-enzymatic antioxidants such as α-tocopherol, ascorbic acid and carotenes complete the antioxidant defence grid [43]. In this review, the discussion will focus on the antioxidant enzymes that may contain manganese in their active centre: SOD, CAT and GPx (Figure 1). The global role of these cellular antioxidant defences is the reduction of ROS to water.

Peer-reviewed version available at Antioxidants 2020, 9, 727; doi:10.3390/antiox9080727

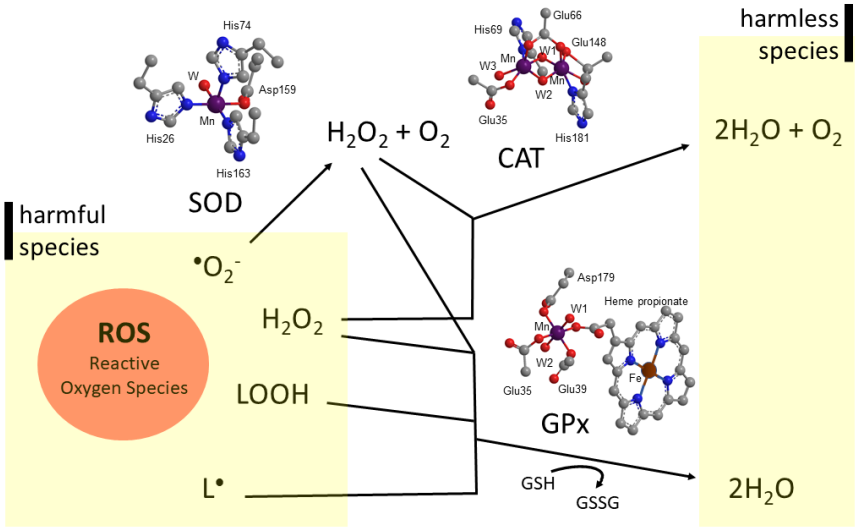

Figure 1. Chemical transformations of some of the ROS harmful species (superoxide radical -$•O_2^-$-, hydrogen peroxide -H_2O_2-, lipid hydroperoxides -LOOH- or lipid radicals -L•) into harmless species through manganese antioxidant enzymes. The figure shows the core of the active site of human mitochondrial SOD2, the core of the active site in *Lactobacillus plantarum* catalase (CAT), and the core of the active site of manganese glutathione peroxidase (GPx). W = water; GSH = monomeric glutathione ; GSSG= glutathione disulfide.

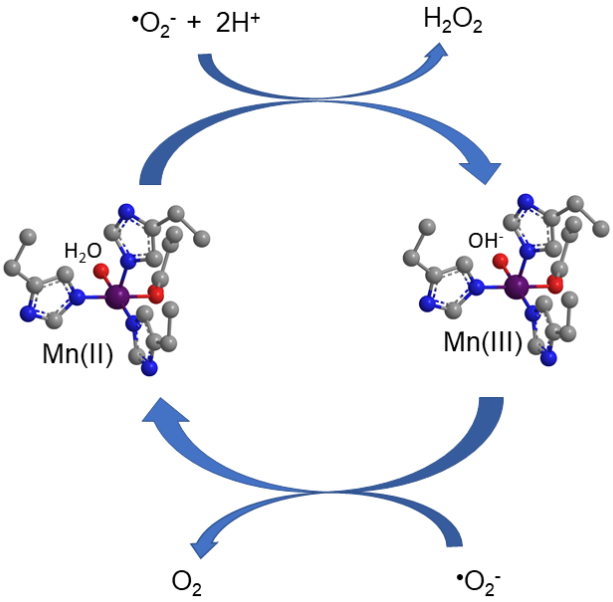

Figure 2. Mechanism of superoxide dismutase activity for superoxide radical anion disproportionation by human manganese SOD2 enzyme.

Superoxide radical anion is a harmful species produced during respiration from the one-electron reduction of molecular oxygen. SOD catalyzes the dismutation of two superoxide anions to yield hydrogen peroxide and molecular oxygen [44]. SODs are classified according to their metal ion cofactor as Fe-SODs, Ni-SODs, CuZn-SODs and MnSODs [45] The SOD2, also known as manganese-dependent superoxide dismutase, is located within the inner mitochondrial matrix, the main site of free radical production from the electron transport chain [46], being pivotal in the ROS release in humans. The coordination environment around the manganese ion for human SOD2 is depicted in

Figure 1. The metal ion shows a trigonal bipyramidal geometry, bound to three histidine ligands (His26, His74 and His163), one aspartate (Asp159) and water or hydroxide as the fifth ligand. These amino acids and ligands constitute the inner sphere with a direct interaction with the manganese. The outer sphere residues are not shown in the figure but they are also essential for efficient dismutation and comprise His30, Tyr34, Phe77, Trp78, Trp123, Gln143, Trp161 and Glu162. The superoxide disproportionation mechanism by SOD2 involves a cyclic one-electron oxidation and reduction of the manganese ion between Mn(II)/Mn(III) oxidation states (Figure 2). The catalysis occurs at a rate approaching the diffusion-controlling region (log $K_{SOD} \sim 10^9$ M^{-1} s^{-1}) [47].

Hydrogen peroxide is generated as a by-product of mitochondrial electron transport of aerobic respiration. As it has been mentioned, it is one of the products of the superoxide radical anion dismutation by SOD. Although H_2O_2 is less reactive than superoxide, control of H_2O_2 levels is also critical [48]. Catalase enzymes catalyse the decomposition of hydrogen peroxide to water and oxygen [49]. The non-heme dinuclear Mn catalase was isolated from bacteria. Figure 1 shows the core of the active site of this enzyme in *Lactobacillus plantarum* [50]. The dimanganese core is bridged by a single carboxylate (Glu66), which spans the cluster. Two single-atom solvent bridges (oxo, hydroxo or aquo) lie between the manganese ions and provide electronic coupling between the two manganese ions. In addition, one of the manganese ions completes its octahedral geometry with a monodentate carboxylate (Glu35), a histidine (His69) and a terminally bound water molecule. The other manganese ion, also displaying an octahedral geometry, is bound to a bidentate (chelating) glutamic acid (Glu148) and a monodentate histidine (His181). The catalase reaction proceeds via a two-electron oxidation-reduction cycle during turnover (Figure 3).

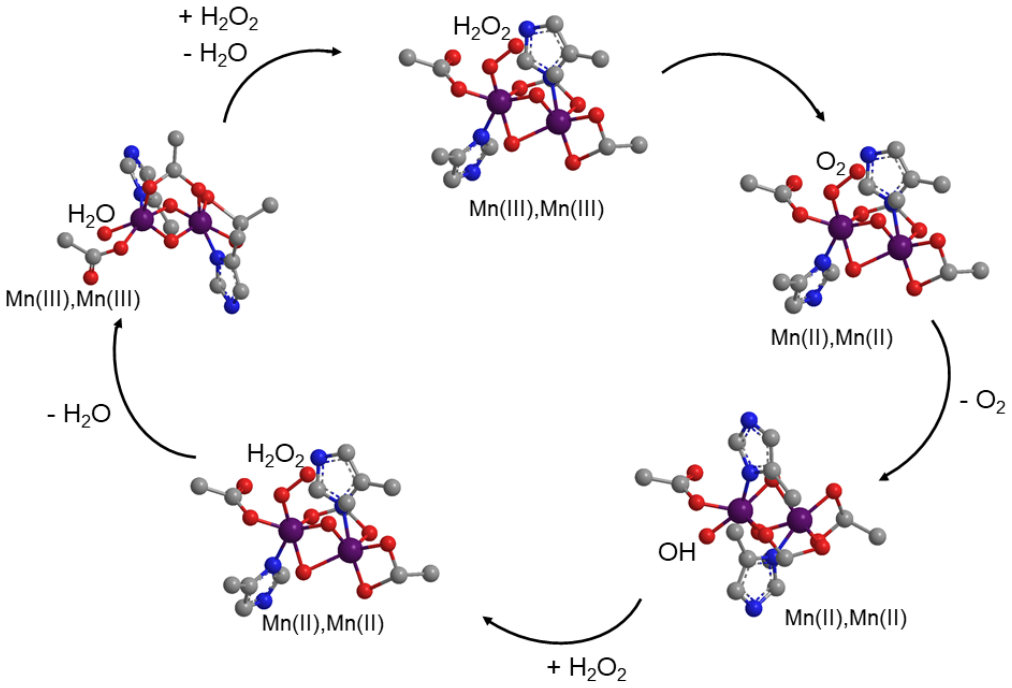

Figure 3. Mechanism of catalase activity for hydrogen peroxide disproportionation by manganese peroxidase enzyme from *Lactobacillus plantarum*.

Peroxidases catalyse the oxidation of a broad range of substrates by hydrogen peroxide [51]. Hydrogen peroxide is then reduced by two electrons using glutathione (GSH) as a sacrificial reductant, yielding water and glutathione disulfide (GSSG). The manganese binding site of the manganese peroxidase is also shown in Figure 1. One heme propionate, two water molecules and the side chains of Glu35, Glu39 and Asp179 have been identified as the manganese(II) ligands in the inner sphere [52], giving rise to an octahedral geometry for the metal ion. GPx also contains a selenocysteine which plays an essential role during the reaction mechanism, since it involves oxidation of the selenol by one molecule of hydrogen peroxide, starting a reaction chain that release GSSG as the by-product

(Figure 4). Peroxidases are not limited to H_2O_2 as the substrate but also catalyse the conversion of organic peroxide to alcohol. Thus, peroxidases can eliminate lipid hydroperoxides, another ROS which contributes to the progression of disbalanced redox homeostasis [53]. Lipid hydroperoxides can be generated very easily in neuronal membranes rich in polyunsaturated fatty acids like arachidonic acid, docosahexaenoic acid, or eicosapentaenoic acid. The peroxidase activity may be successfully be used for different industrial processes since peroxidases are capable of degrading a broad range of chemical structures as lignin or different recalcitrant pollutants like polycyclic aromatic hydrocarbons [54].

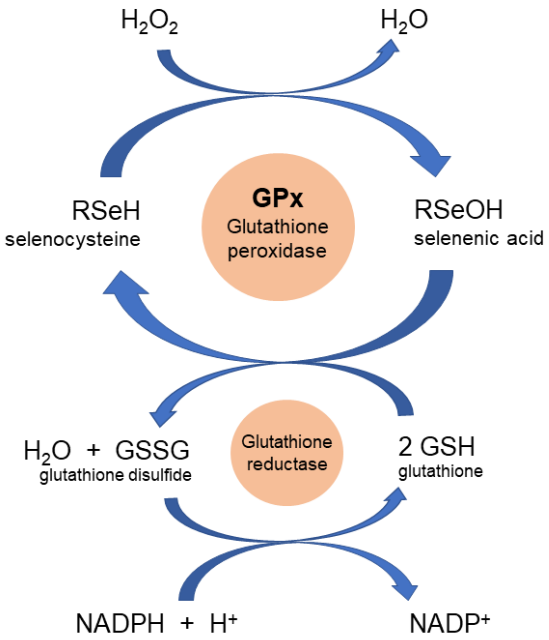

Figure 4. Mechanism of peroxidase activity for hydrogen peroxide reduction by the native glutathione peroxidase enzyme.

The enzymatic antioxidant defence along with other non-enzimatic antioxidants act in an effective way against free radicals and other reactive oxygen species to control their damaging effects to macromolecules and body tissues. However, overproduction of ROS leads to oxidative damage of cellular structures due to an imbalance in the oxidant-antioxidant status [55]. ROS excess may be derived from exogenous sources as mentioned above, but also by faulty regulation of cellular antioxidant defences, normally associated with aging, that can lead to accumulation of toxic levels of ROS [56]. For this situation, a strategy to recover a balance between ROS generation and removal is the use of antioxidants as therapy [57-60]. Administration of exogenous native antioxidant enzymes has not been successful for therapeutic treatment of oxidative stress because of several limitations [61]: i) short half-life of the enzymes; ii) difficulty to enter in the cells due to their high molecular weight; iii) antigenicity; iv) high-manufacturing costs. To overcome these limitations, pharmacological research has pointed at the development of low molecular weight SOD/CAT mimics [62-65]. Several supplements as α-tocopherol [66], ascorbic acid [67], carotenes [68-69] or other organic molecules [70-73] with antioxidant properties have been also used for the therapeutic treatment of oxidative stress-induced diseases.

3. Manganosalen complexes as catalytic antioxidants

Manganosalen complexes are coordination compounds with a chelate bis-Schiff base ligand obtained by condensation of salicylaldehyde and a diamine. The acronym *salen* is due to the reactants used to synthezise the ligand N,N-bis(salicylidene)ethylendiamine, obtained by condensation of salicylaldehyde (sal) and ethylenediamine (en) [74]. Although the latter ligand is strictly speaking the salen ligand, actually the class of compounds known as manganosalen complexes encompasses other

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 July 2020 doi:10.20944/preprints202007.0222.v1

Peer-reviewed version available at Antioxidants 2020, 9, 727; doi:10.3390/antiox9080727

bis-Schiff base ligands (containing two bisimine groups), obtained from different diamines (propylenediamine, phenylenediamine, butylendiamine, etc.) as well as the group of their derivatives with different substituents both in the phenyl rings and in the diamine spacer [20,75]. Figure 5 collects some manganosalen complexes with pharmacological relevance used in *in vivo* studies, corresponding to the EUK series patented by Eukarion.

This type of ligands has oxygen and nitrogen donor groups, which decrease the Mn(III)/Mn(II) redox potentials upon coordination (E^{o} = 1.51 V for $[Mn(H_2O)_6]^{3+}$, a rather oxidizing potential), and the resulting complexes constitute suitable systems to catalyse multiple redox reactions [20,75-79]. Redox potentials of manganosalen complexes may be tuned by modifying other features than these substituents. In this sense, the number of ligands or the geometry around the metal ion are factors that influence the manganese redox potential of the final complex. For example, alkoxy substituents in the phenyl rings, particularly 3-methoxy or 3-ethoxy groups (see EUK-113, EUK-134, EUK-172, EUK-178 or EUK-189 in Figure 5), lowers the redox potentials for the manganosalen complexes due to the electron-donor character of these substituents, thus facilitating to achieve higher oxidation states for the manganese ion during the enzymatic activity.

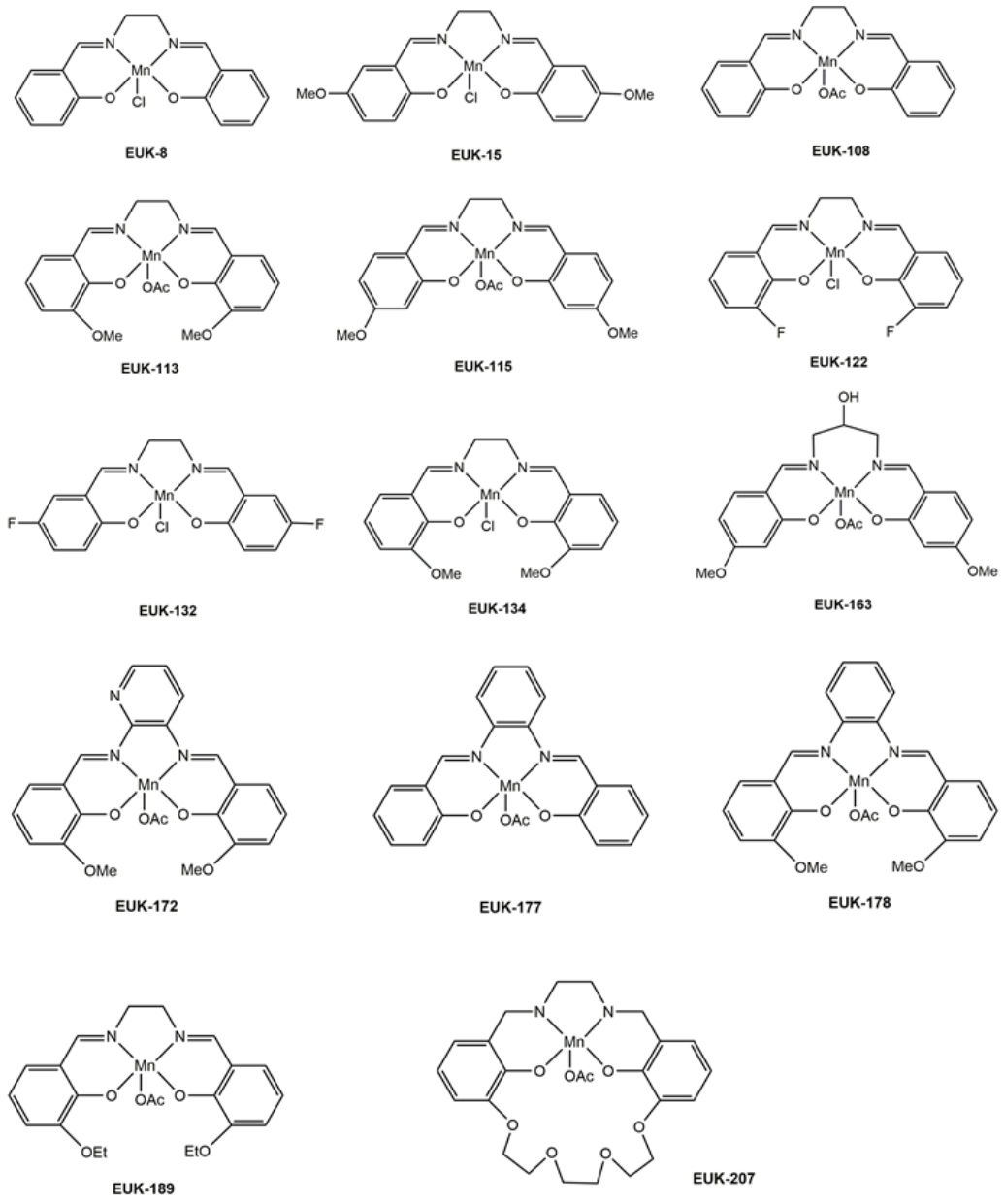

Figure 5. Structures of some manganosalen complexes, corresponding to the EUK series, with pharmacological relevance.

The chelating bis-Schiff base ligand forms a typical almost planar MnN_2O_2 core where the 5- or 6-member chelate rings confer high stability. The introduction of different auxiliary ligands (halides, carboxylates, alcohols, dicyanamides, thiocyanates, etc.) alters the geometry of the compounds [20,80], giving rise to different behaviours in catalysis. Labile auxiliary ligands or solvent molecules in the axial positions favour catalytic activity through an inner-sphere electron transfer mechanism in which a vacant can be generated in this site, where the substrate molecule can be subsequently accommodated [81]. A short two-carbon chain between the imine groups in the Schiff base ligand constricts the chelate ring once the nitrogen atom coordinates to the metal, leading to tetragonally elongated geometries [23,81-84]. On the contrary, if the Schiff base ligand has a flexible three-membered alkyl chain between the imine groups, a better stabilization of a high-symmetry octahedral symmetry is achieved, and subsequently the generation of a coordination site gets difficult. Thus, a correlation between the factor of the tetragonal elongation and catalytic activity as enzyme mimics has been reported for manganosalen complexes [23,81-84].

Supramolecular interactions may play different crucial roles in enhancing the activity as enzyme mimics. On the one hand, hydrogen bonding and other supramolecular contacts may induce self-organization of the complexes to afford dimeric entities [85]. Thus, these supramolecular mechanisms may allow aggregation of the complexes into dimers once the monomers cross the cell membrane. On the other hand, supramolecular interactions also play an essential role in biological processes recognition [86]. Second-sphere effects of the substituents may also modulate the redox potentials of the metal centre and the metal-ligand bond strength and guide their reactivity with superoxide anion radical [87]. Alkoxy substituents on the phenyl rings can also participate in establishing supramolecular interactions through hydrogen bonding, which added to their mentioned effect on redox potentials, could explain the high activity as enzyme mimics of alkoxy substituted manganosalen complexes [88-90].

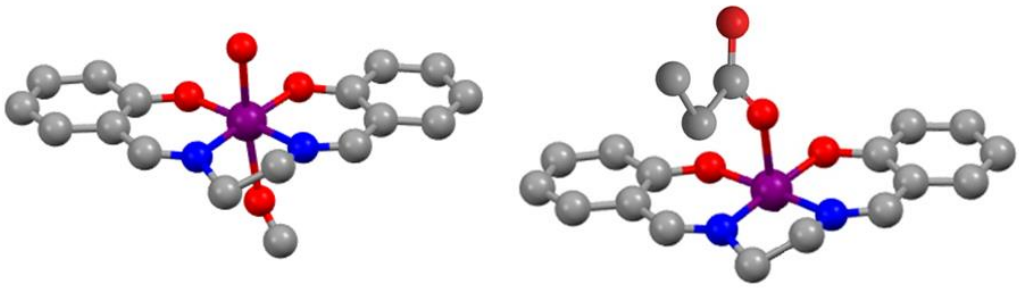

Figure 6. Manganosalen model complexes with distorted octahedral geometry (left, coordination number 6) or distorted square-planar pyramidal geometry (right, coordination number 5).

The relatively planar MnN_2O_2 core of manganosalen complexes is somewhat similar to that of natural manganese Mn-macrocycles or Mn-porphirins, and this similarity is shown in their chemical reactivity. However, the manganese ion in manganosalen compounds is coordinated to oxygen and nitrogen atoms, which contrasts with porphyrins where the metal is coordinated to nitrogen atoms only. Oxygen and nitrogen are the most common donor atoms in biological systems, so that the structure of manganosalen complexes (Figure 6) resembles those of various native manganoenzymes. Manganosalen compounds are easily obtained from inexpensive precursors, and they are cheaper than Mn-porphirins.

One of the most interesting properties of the manganosalen complexes is that they are cell-permeable, with better bioavailability than exogenous antioxidant enzymes [91-92]. Manganosalen complexes exhibit high SOD, catalase and peroxidase activities, which has led to their development as catalytic antioxidants [6-10,13,18-21,23], a term used for all cases in which one single molecule of catalyst induces the detoxification of numerous ROS molecules.

Malfroy *et al.* first reported the SOD mimetic properties of manganosalen complexes [93]. Based on stopped-flow analysis combined with time-resolved UV/vis spectroscopy and global spectra

Peer-reviewed version available at Antioxidants 2020, 9, 727; doi:10.3390/antiox9080727

analysis of superoxide decay, it has been reported that manganosalen complexes possess a SOD activity of about 2 x 10^6 M^{-1} s^{-1} [94-95], both in Hepes and in phosphate buffers. The mechanism followed by the manganosalen complexes is similar to that of the native SOD enzyme, a ping-pong mechanism where a superoxide anion radical reduces the synthetic manganese complex from Mn(III) to Mn(II), which is subsequently oxidizes back to Mn(III) by a second superoxide anion radical (Figure 7) [10,19,23].

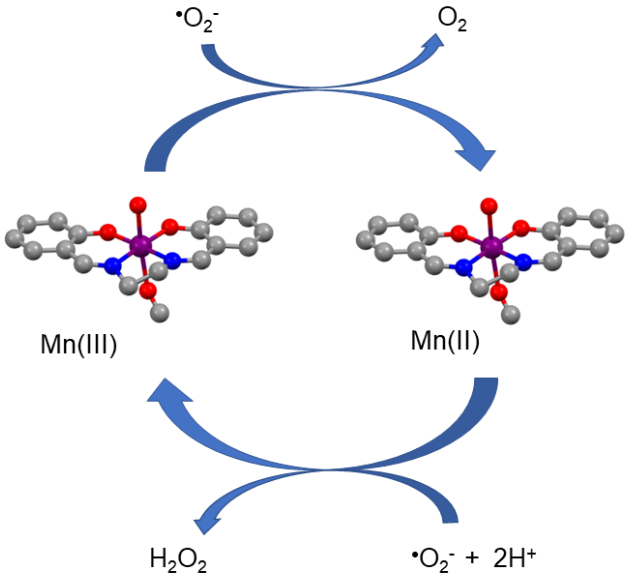

Figure 7. Mechanism of superoxide dismutase activity for superoxide radical anion disproportionation by manganosalen complexes.

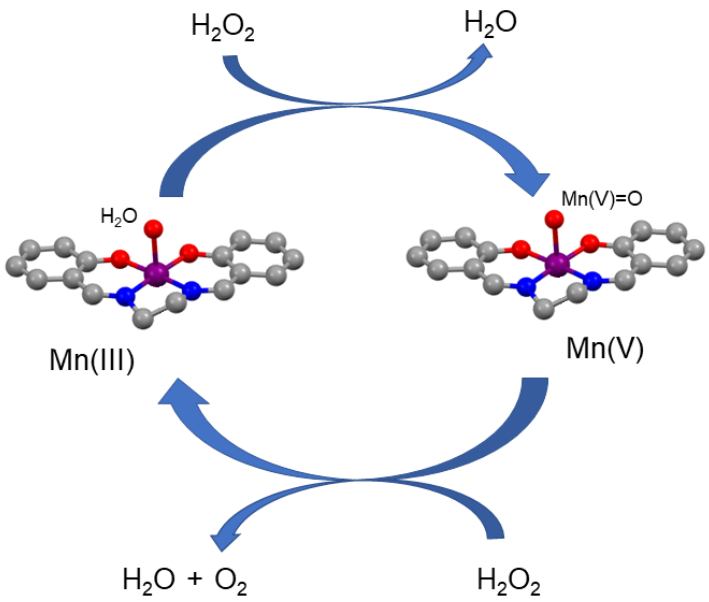

Figure 8. Proposed mechanism of catalase activity for hydrogen peroxide disproportionation by manganosalen complexes.

While the SOD activity of manganosalen complexes hardly varies with derivatization, their catalase activity is highly sensitive to the substituents on the aromatic rings or the length of the alkyl chain in the spacer between the imine groups [90], ranging from inactivity to high efficiencies in the hydrogen peroxide disproportionation [81-84,96]. The efficiency of these systems seems to be related

Peer-reviewed version available at *Antioxidants* **2020**, *9*, 727; doi:10.3390/antiox9080727

to the presence of at least one vacancy or a labile coordination position on the manganese core. Electron donor substituents on the phenyl rings of the salen moiety also increase the catalase activity of manganosalen complexes [87]. The rates at which manganosalen complexes scavenge hydrogen peroxide are similar to those reported for metalloporphyrins. For instance, compound EUK-172 (see Figure 5) is reported to have a catalase rate greater than 1 mM O_2/min [96], measured by monitoring the conversion of hydrogen peroxide to oxygen using a Clark type oxygen electrode [97].

The mechanism of these synthetic mimics is proposed to involve mononuclear Mn(V)=O species [98-99] (Figure 8), although some mimics may follow a mechanism through the formation of dimeric species in solution [81]. Anyhow the mechanism is different from the native catalase enzyme shown in Figure 3.

Peroxidase mimics are able to convert H_2O_2 to H_2O but also to scavenge other peroxides, including lipid hydroperoxides or other organic peroxides [96,100], so that they could scavenge lipid peroxides in tissues. The peroxidase activity of manganosalen complexes is related to achieve square pyramidal or tetragonally elongated octahedral geometries [81,84], facilitating substrate coordination. In this way, as previously mentioned for other catalytic activities, manganese complexes with an n-propyl spacer between the imine groups show little or no activity, whereas short two-carbon chains enhance the peroxidase activity. Electronic effects contributions by the substituents on the phenyl rings tune the stability of the oxidation states and modulate the reversibility of the catalytic action. Manganosalen complexes are proposed to behave as peroxidase mimics by a mechanism quite similar to that of their catalase action, through a Mn(V)=O intermediate, which is able to oxidize an organic substrate (Figure 9) to afford the initial catalyst complex.

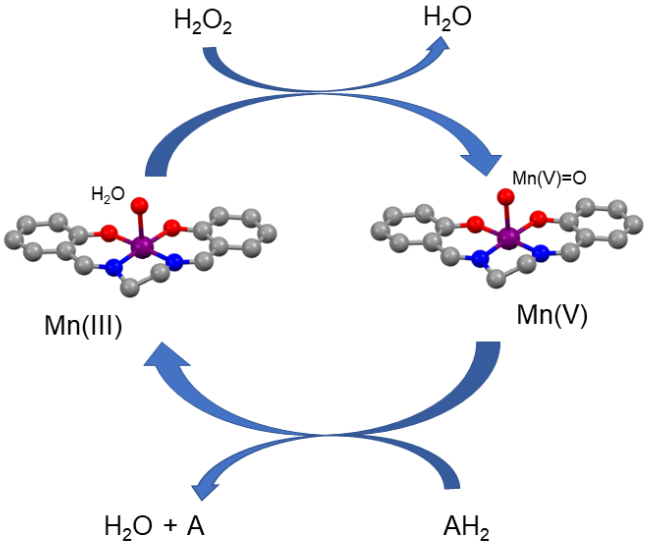

Figure 9. Proposed mechanism of peroxidase activity for manganosalen complexes (AH_2 is an oxidizable substrate).

Manganosalen complexes often have multiple antioxidant activities at the same time, showing, for instance, both SOD and catalase functions. This reactivity against different ROS is attractive since hydrogen peroxide is a product released by the SOD activity. However, recent findings open other interesting antioxidant routes, like a cascade mechanism, where the initial complex only has SOD activity but not catalase function [10,19]. In this case, the catalase active complex is activated after radical scavenger reaction, so potential redox side effects within the cell can be avoided.

4. Therapeutic effects of manganosalen complexes in *in vivo* models

The SOD, catalase and peroxidase activities shown by manganosalen complexes have attracted attention for their use as catalytic antioxidants. Subsequent *in vitro* studies have confirmed their

Peer-reviewed version available at Antioxidants 2020, 9, 727; doi:10.3390/antiox9080727

efficiency in oxidative stress models [6,8,9-10,13-15,20-21,23 and other references cited therein], although the focus of this review is the evaluation of the *in vivo* studies with different animal models. These studies are organized below according to the pathology or the oxidative damage produced by ROS.

4.1. Neurodegenerative diseases and mental disorders

The brain is prone to oxidative stress as a result of the high levels of oxygen required, which represents 20% of oxygen uptake when brain accounts for only 2% of body weight. Neurons consume a high rate of energy (4×10^{12} ATP/minute) meaning large amounts of oxygen to maintain neural intracellular ion homeostasis [41]. Moreover, neural membranes have high concentrations of polyunsaturated fatty acids which generate lipid hydroperoxides. Additional factors, as the presence of autooxidizable neurotransmitters, increase the sensitivity of this organ to ROS-mediated damage. Excessive ROS levels have been associated with neurodegenerative disorders like Alzheimer's Disease (AD), Parkinson's Disease (PD), amyotrophic lateral sclerosis (ALS) or Huntington's Disease [42].

As previously mentioned, EUK-8, EUK-134 and EUK-189 models were injected to SOD2 nullizygous mice to rescue them from oxidative neurodegenerative process [4]. These compounds had previously shown efficacy in a variety of oxidative stress paradigms [101-103]. For instance, EUK-134 had found to protect most of the vulnerable neurons from excitotoxic cell death in Sprague-Dawley rats [102]. Administration of these three manganosalen compounds extended the lifespan of the mice lacking SOD2 approximately threefold and eliminated clinical signs of the associated neurobehavioral phenotype previously described. This study also indicate that these catalytic antioxidants can cross the blood-brain barrier, particularly EUK-189, which is slightly more lipophilic than EUK-134. In a later study, using the same animal model, Melov *et al.* reported that neural cell death in defined regions of the frontal cortex of SOD2 null mice is a consequence of endogenous mitochondrial oxidative stress [104]. In this study, they could partially rescue neural cell death by treatment with a high dose of EUK-8. Melov *et al.* also reported the therapeutic effects of EUK-189 in preventing a neurodegenerative phenotype in an Ah-transgenic mouse model for Alzheimer disease [105]. This manganese complex and the cyclic analogue EUK-207 were used in a study with C57BL/6N Sim middle-age mice [106], which usually exhibit a dramatic decrease in learning and memory function between 8 and 11 months of age, associated with oxidative protein damage in the brain [107]. Treatment during a 3-month period with EUK-189 or EUK-207 resulted in an almost complete reversal of age-related learning and memory deficit, showing a complete reversal in protein oxidation and a 50% reduction in age-related increase in lipid peroxidation. EUK-207 exhibits longer plasma half-life than EUK-189, and subsequently greater biological stability. Two such molecules were also used by Baudry *et al.* in older mice, at a lower dose, and for longer periods of time [108], preventing age-dependent cognitive decline in the same way as previously found for middle-aged individuals. Their results indicate that the age-associated deficits in learning and memory might be originated by oxidative damage to hippocampus, amygdala, or both.

EUK-8 was the first manganosalen complex that showed efficacy for the treatment of a neurodegenerative disease using an animal model (see Table 1). Malfroy *et al.* reported in 1997 [109] how this synthetic catalytic scavenger reduces the severity of autoimmune encephalomyelitis in guinea pigs. Watanabe *et al.* [110] used the same compound to treat small bowel ischemia/reperfusion injury in Sprague-Dawley rats. They compared the protective effects of EUK-8 and a Mn(III)-porphirin SOD mimic, manganese-meso-tetrakis(N-methylpyridinium-2-yl)porphyrin [111]. Both manganosalen compound and Mn-porphyrin showed similar beneficial properties by the inhibition of O_2, H_2O_2, and NO production.

Xu *et al.* [112] reported that EUK-8 and EUK-134 reduced the levels of oxidative stress and prolonged survival in a mouse amyotrophic lateral sclerosis model. In a later study EUK-134 afforded best results than EUK-8 in reducing brain infarct size after middle artery occlusion in a rat model [113]. After the neuroprotective effects shown by EUK-8 and EUK-134, Doctrow *et al.* [86] compared the *in vivo* antioxidant activity of different analogues, including the two cited compounds, EUK-113,

Peer-reviewed version available at *Antioxidants* **2020**, *9*, 727; doi:10.3390/antiox9080727

EUK-161, EUK-163, EUK-172, EUK-178 and EUK-189 in a rat stroke model. They concluded that alkoxy substituents on the phenyl rings for this series of compounds confers some advantage toward the biological protective effects of these manganosalen complexes.

Table 1. Selected *in vivo* trials of manganosalen complexes treatments for combating neurodegenerative diseases.

Antioxidant model	Disease	Animal model	Dose	Outcomes	Ref.
EUK-8 EUK-134 EUK-189	Spongiform neurodegenerative disorder	SOD2 nullizygous mice	30 mg/kg	Rescue of the neurodegenerative disorder	4
EUK-207	Age-related cochlear cell degeneration	SAMP8/SAMR1 mice	0.2 mg/kg/day	Prevention of age-related hearing loss	22
EUK-8	Ischemia/reperfusion injury	Sprague-Dawley rats	1 mg/Kg, 2 times	Protective effects	92
EUK-134	Kainite-induced neuropathology	Sprague-Dawley rats	10 mg/Kg, 2 times	Prevention of excitotoxic neuronal injury	102
EUK-8	Autoimmune encephalomyelitis	Guinea pig MBP	100 mg/Kg	Prevention and suppression of the disease	103
EUK-8	Neurodegeneration	SOD2 nullizygous mice	30 mg/kg	Rescue of neuronal cell death	104
EUK-189	Human prion disease	Balb/c mice	30 mg/kg, 3 t/w[1]	Modest prolong.[2] survival. Reduc.[3] in oxidative damage to proteins.	115
EUK-189	Alzheimer disease	Tg2576 mice	30 mg/kg, 3 t/w	Amelioration of cataracts in the lenses	105
EUK-189 EUK-207	Loss of learning and memory function	C57BL/6N Sim mice	9 nmol/day-0.09 µM/day	Reversion of cognitive deficits	107
EUK-189 EUK-207	Age-related cognitive deficits	C57BL/6N Sim mice	15-16 µg/Kg/day	Reduc.[3] of the age-related cognitive impairment	108
EUK-8 EUK-134	Amyotrophic lateral sclerosis	SOD1-G93A mice	33 mg/Kg, 3 t/w	Prolongation of survival	112
EUK-8 EUK-134	Ischemic brain injury	Sprague-Dawley rats	30 mg/Kg, 3 t/w	Reduction of brain infarct size	113
EUK-134 EUK-139	Dopaminergic neurons death by neurotoxic	C57BL/6 mice	15 mM 1 day prior to the toxic	Attenuated the loss of nigral dopamine neurons	114
EUK-189	Ataxia-telangiectasia	Mice lacking ATM gene	1.2 mg/Kg/day	Correction of the neurobehaviroral abnormality	116
EUK-207	Radiation-induce cognitive impairments	C57Bl6/J mice	0.2 mg/kg/day	Mitigation of the cognitive injury	117

[1] t/w: times a week; [2] prolong.: prolongation; [3] reduc.: reduction

Peer-reviewed version available at Antioxidants 2020, 9, 727; doi:10.3390/antiox9080727

Andersen *et al.* [114] demonstrated the efficacy of EUK-134 and EUK-189 in protecting against paraquat-induced dopaminergic cell death in adult mice *via* inhibition of the activation of JNK-mediated apoptosis. Paraquat is an herbicide that induces selective loss of dopaminergic neurons of the substantia nigra. These two manganosalen complexes significantly inhibited caspase-3 activation, cell death, and DNA fragmentation in in vitro paraquat exposed rat cells. EUK-189 was also employed in a mouse model of human prion disease [115], giving place to a modest 5% increase of survival compared to untreated disease controls. This beneficial effect was correlated with reductions in oxidative, especially nitrative, damage to proteins. The same manganosalen complex, EUK-189, was used by Levine *et al.* [116] to treat the neurobehavioral defect in ataxia-telangiectasia mice, showing that this catalytic antioxidant corrected the neurobehavioral abnormality. This effect, that was reproducible over time, and it involved a reduction in the oxidation of brain fatty acids and also a retard development of thymomas.

More recent studies were focused on the manganosalen EUK-207, due to both its ability to supress oxidative stress and its greater biological stability. Thus, Raber *et al.* [117] reported that this compound mitigated radiation-induced cognitive impairments without affecting cognition of sham-irradiated mice. Wang *et al.* [22,118] used EUK-207 to treat hydrogen peroxide induced DNA damage and senescence phenotype in senescence-accelerated mouse-prone 8 mice, reducing age-related loss of both hearing and hair cells degeneration. In their study, they found that cochleae cells treated with EUK-207 displayed increased levels of FOXO3a and Nrf2, two transcription factors that have been previously shown to positively regulate cellular resistance to oxidative stress [119-120].

4.2. Inflammatory diseases

The neuroprotective effects shown by manganosalen complexes are close related to their ability to attenuate inflammation makers such as cytokines or chemokimes. High amounts of hydrogen peroxide activate a number of transcription factors as NFkB, AP-1 and Nrf2 [121], whose levels may be regulated by the administration of manganosalen complexes. The anti-inflammatory effects of these catalytic antioxidants play a beneficial role not only in neurons or in the respiratory system, but also in a wide range of pathologies related to inflammation (Table 2).

Hill *et al.* [122] treated Sprague-Dawley rats with EUK-189 to mitigate the DNA damage in rat lung after exposed to 10-20.5 Gy doses of gamma rays. The catalytic antioxidant resulted effective at reducing micronucleus formation in lung fibroblasts and this could be attributed to the ability of this compound to suppress the signal that would normally turn on the inflammatory response to repair the radiation insult.

EUK-134 was used by Lawler *et al.* [123] as ROS scavenger in myopathy in the diaphragm of the *mdx* mouse model. This manganosalen complex reduced hydroperoxides, markers of oxidative stress in this muscle, attenuated both by the elevation of inflammatory cell invasion and the NF-κB activity and p65 subunit protein levels in the *mdx* diaphragm. Muzykantov *et al.* [124] employed PEG-liposomes loaded with EUK-134 to alleviate acute pulmonary inflammation induced by endotoxin in mice. This manganosalen compound was also used by Singh *et al.* [125] to mitigate zinc- and paraquat-induced toxicity in rat polymorphonuclear leukocytes.

Other manganosalen compounds were used with efficacy against inflammatory episodes is EUK-207. Hill *et al.* [126] reported that this synthetic model mitigated the radiation-induced lung injury in 6- to 7-week-old Fisher rats, by scavenging ROS and reducing activity of the NFkB pathway. In this study, EUK-207 also showed the ability to reduce levels of TGF-β1 expression, activated macrophages and fibrosis.

A study that deserves a more detailed analysis is the carried out by Kash *et al.* [127] with EUK-207 to reduce lung damage and to increase survival during 1918 influenza virus infection in mice. This pandemic, caused by the H1N1 influenza A virus, infected at about a third of the world's population of 1918, and resulted in 40-60 million deaths worldwide. This influenza and SARS-CoV-2 share some similarities in the way they may lead to a respiratory failure. Many studies demonstrated that the severe lung pathology provoked by the 1918 influenza virus infection was associated with immunopathogenic immune response with excessive inflammatory and cell death

Peer-reviewed version available at Antioxidants 2020, 9, 727; doi:10.3390/antiox9080727

responses [128]. EUK-207 treatment caused a marked reduction in the severity of lung pathology and substantially reduced cell death responses at both the RNA and the protein levels. The beneficial effect of this manganosalen complex in an animal model affected by the 1918 influenza is related to its ability to reduce the most relevant cytotoxic effects of ROS, thereby limiting excess of cell death responses and allowing for increased lung repair responses. As the antioxidant effects of EUK-207 regulate the response of the host, it could be a therapeutic alternative to other organism's infections than influenza. In this sense, it would be interesting to test this hypothesis with studies in animal models of the response to combined treatments of manganosalen complexes and antiviral against the SARS-CoV-2 virus.

Table 2. Selected *in vivo* trials of manganosalen complexes treatments for combating inflammatory diseases.

Antioxidant model	Disease	Animal model	Dose	Outcomes	Ref.
EUK-189	Radiation-induced lung injury	Sprague-Dawley rats	30 mg/kg	Reduction micronucleous formation in lung fibroblasts	122
EUK-134	Proinflammatory damage in the diaphragm muscle	Mdx mice	30 mg/kg/day	Reduction of muscle damage	123
EUK-134	Acute pulmonary inflammation	C57BL/6 mice	200 µL aliquots of 3.2 mg total lipid at 2000 CPM/µL	Alleviate acute pulmonary inflammation	124
EUK-134	Paraquat-induced inflammation	Wistar rats	10 mg/kg	Protection from the toxic effects	125
EUK-207	Radiation-induced lung injury	Fisher rats	8 mg/kg	Mitigation of lung injury	126
EUK-207	1918 influenza virus	BALB/c mice	30 µg/day	Reduction of severity lung injury	127
EUK-8	Acute lung injury	Pigs	10 mg/kg bolus and 3 mg/kg.h, n = 6	Alleviate acute lung injury	129-131
EUK-207	Radiation-induced lung damage	Sprague-Dawley rats	8 mg/kg/day	Limitation of pulmonary fibrosis	133-134
EUK-207	Radiation-induced lung injury	Sprague-Dawley rats	8 mg/kg/day	Mitigation of the radiation effects	135

Different previous studies showed the beneficial effects of manganosalen complexes to treat lung injuries. EUK-8 was used by Fink *et al.* to attenuate many of the features of LPS-induced acute lung injury in a porcine model [129-131], by detoxifying ROS without affecting the release of other important proinflammatory mediators like 6-keto-prostaglandin F1 alpha, thromboxane B2 or tumor necrosis factor alpha. EUK-134 was the antioxidant catalytic model chosen by Kamp *et al.* [132] to alleviate asbestos- and H_2O_2-induced damage in mice, limiting pulmonary fibrosis. Hill *et al.* [133-134] reported the mitigation of radiation-induced lung injury by EUK-207 in Sprague-Dawley rats. Treatment with this manganese-model decreased hydroxyproline content, 8-hydroxy-2-

Peer-reviewed version available at Antioxidants 2020, 9, 727; doi:10.3390/antiox9080727

deoxyguanosine, malondialdehyde levels, and activated macrophages levels. Lung levels of the cytokine transforming growth factor-β1 also decreased. Medhora *et al.* [135] reported the antioxidant effect of EUK-207 to reduce pneumonitis and pulmonary fibrosis after thoracic irradiation in a rat model.

The anti-inflammatory effect of other catalytic antioxidants, inspired by manganosalen complexes, but not belonging to the EUK series, has also been studied. A bioinspired manganese SOD mimic, reported by Policar *et al.* [136], demonstrated efficiency as anti-inflammatory agent for C57BL/6 mice with DNBS-induced colitis.

4.3. Cardiovascular diseases

Inflammation and tissue damage are closely related to different pathologies, including some cardiovascular diseases. Inflammation is common for heart disease and stroke patients. The role of excessive ROS production during haemorrhagic shock and reperfusion injury has been well documented [137]. In this way, some already cited studies of manganosalen complexes treatments for anti-inflammatory models, also showed beneficial effects for cardiovascular diseases (Table 3). For instance, the commented *in vivo* study of Medhora *et al.* [135] with EUK-207 mitigated multiple vascular injuries in irradiated lungs.

Table 3. Selected *in vivo* trials of manganosalen complexes treatments for combating cardiovascular diseases.

Antioxidant model	Disease	Animal model	Dose	Outcomes	Ref.
EUK-8	Pressure overload-induced heart failure	B6CBA mice hemizygous or homozygous for the X-linked Hq mutation	25 mg/kg/day	Prevention myocardial damage. Attenuation cardiac hypertrophy and fibrosis	138
EUK-189	Gamma irradiation	C3H/HeN and CD2F1 mice	70 mg/kg	Increase of 30-day survival	139
EUK-134	Pulmonary hypertension	Wistar rats	3 mg/kg/day	Prevention of diaphragm muscle weakness	141
EUK-207	Atherosclerosis	C57B1/6 mice	1 mg/kg/day	Reduction for endothelial-associated events	142

De Windt et al [138] used EUK-8 to reduce cardiac oxidative stress in Harlequin mutant mice and their wild-type counterparts. The results of this study showed an improvement of the left ventricular end-systolic dimensions and a fractional shortening by using this manganese complex. EUK-8 also attenuated necrotic and apoptotic cell death, prevented myocardial oxidant stress, and attenuated cardiac hypertrophy and fibrosis.

EUK-189 increased 30-day survival in irradiated mice according to a study by Whitnall *et al.* [139]. This manganosalen complex increase the number diverse circulating blood elements like total white blood cells, lymphocytes, eosinophils, and platelets. The same catalytic antioxidant was tested by Monsalve *et al.* [140] to regulate ROS homeostasis and to control the vascular endothelial cells function in mice. EUK-189 restored endothelial growth factor-A signalling in peroxisome proliferator activated receptor γ co-activator 1α (PGC-1 α), a process to be relevant in metabolic disorders where

Peer-reviewed version available at Antioxidants 2020, 9, 727; doi:10.3390/antiox9080727

microvascular complications are frequent, like diabetic retinopathy. Excessive ROS appeared as key factor in the alteration of the endothelial growth factor-A signalling and in the capacity of endothelial cells to form stable interactions with other endothelial cells and with the extracellular matrix, but these alterations were partially reversed by administration of EUK-189.

Recent studies have shown the efficacy of other manganosalen complexes for the treatment of different cardiovascular models. Yamada *el al.* [141] reported that EUK-134 prevented the force decrease and the actin modifications in pulmonary hypertension diaphragm bundles in Wistar rats. In their study they found that this manganosalen complex does not alter diaphragm contractile function in normal rats. Lindner *et al.* [142] evaluated the therapeutic effects of EUK-207 in mice with age-dependent atherosclerosis. Long-duration therapy (40 weeks) with EUK-207 almost completely suppressed plaque development and macrophage content in thoracic aorta of the treated mice compared with control mice. However, therapy for eight weeks did not affect the area or the macrophage content.

4.4. Skin damage

In the same way as discussed for endothelial cells, any tissue, including the skin, can suffer oxidative damage both of inflammatory and non-inflammatory origins. Several studies have focused on the protective effects of the manganosalen compounds in ROS-mediated skin damage (Table 4). Skin is exposed to solar ultraviolet irradiation, ozone, smoke and air pollution. All of them are environmental sources of ROS that induce damage to lipids, proteins and DNA, playing a role in the skin aging process [143].

Table 4. Selected *in vivo* trials of manganosalen complexes treatments in ROS-mediated skin damage.

Antioxidant model	Disease	Animal model	Dose	Outcomes	Ref.
EUK-8 EUK-134 EUK-189	Rejection of allogeneic skin grafts	BALB/c and C57BL/6 mice	25 mg/kg/day	Attenuation on graft rejection	144
EUK-134	UV-induced skin damage	Humans volunteers	Topical 0.01-0.1%, 3 μ L cm^{-2}	Protection of skin surface from accumulating oxidative damage	12
EUK-207	Radiation dermatitis	WAG/RijCmcr mice	1.8 mg/kg/day	Promotion wound healing in irradiated skin	147
EUK-207	Radiation-induced DNA damage or lipid peroxidation	C3H/HeJ mice	30 mg/kg	Protection before irradiation. Mitigation of lipid peroxidation	148

EUK-8, EUK-134 and EUK-189 were used by Benichou et al. [144] to delay the rejection of fully allogeneic skin transplants in mice. Mice treated with EUK-189 showed the longest skin graft survival, and along with EUK-134, exhibited the longest delays of graft rejection. The three manganosalen complexes reduced anti-donor cytotoxic responses in skin-grafted mice, and they decreased pro-inflammatory type 1 alloresponse while promoted anti-inflammatory type 2 alloimmunity.

Declercq et al [12] carried out a study of the protective effects of EUK-134 on the human skin of 748 healthy volunteers (18-80 years of age) over a period of 4 years. EUK-134 had been previously reported to increase cell survival in normal human keratinocytes upon exposure to ultraviolet-B, superoxide or hydrogen peroxide [145-146]. In the study with the human volunteers, EUK-134 (applied at a concentration of 0.01-0.1%) reduced the level of skin surface lipid peroxidation in UVA

Peer-reviewed version available at Antioxidants 2020, 9, 727; doi:10.3390/antiox9080727

exposed skin. Noteworthy that the reduction of squalene hydroperoxide levels at the skin surface was found even when applied the antioxidant after UVA-exposure. As a consequence of this study EUK-134 is now commercially available as an antioxidant for the protection of dry or irritated skin.

EUK-207 was tested in a study performed by Lazar *et al.* [147] as a potential mitigating drug on end points relevant to radiation dermatitis, skin wound healing, and chronic oxidative stress in rats. The EUK-207-treated mice group showed reduced radiation dermatitis severity by 30 days after irradiation and displayed significantly smaller wounds than vehicle-treated rats. Thiss manganosalen complex also reversed and normalized the gene expression pattern in irradiated skin by reducing the oxidation of proteins and nucleic acids. The same compound was used by Hill *et al.* [148] to mitigate the radiation-induced DNA damage and the lipid peroxidation in mice. They found that EUK-207 provided some protection against DNA damage only when was delivered before irradiation. They also demonstrated significant protecting effects on radiation-induced lipid peroxidation at one or more of the three time points after local skin irradiation.

4.5. Fetal malformations

Pregnancy is a state of oxidative stress due to high metabolic activity in the fetoplacental compartment. Regulation of ROS during gestation is a complex process, whereas excessive oxidant levels cause biomolecules damage and leads to fetal malformations as a consequence of the attack by ROS formed during the resumption of placental perfusion. On the other hand, the maintenance of a physiological level of oxidant levels is essential for governing life processes through redox signalling [149]. Two studies have been reported about the fetal protection or the reduction of pregnancy complications by manganosalen complexes.

Zhang *et al.* [150] studied the effect of long-term high-altitude hypoxia (a severe lack of oxygen) during gestation in sheep. Uterine arteries of pregnant sheep are affected by chronic hypoxia due to an inhibition effect of the large conductance Ca^{2+} activated K^+ (BK_{Ca}) channel activity by increasing oxidative stress. Treatment of the pregnancy sheep with EUK-134 resulted in a mitigation of the hypoxia effects on BK_{Ca} channel currents in uterine arteries, alleviating pregnancy complications such as pre-eclampsia and fetal growth restriction.

The same manganosalen complex was used by Chen *et al.* [151] to protect ethanol-induced limb malformations in mice. In vivo treatment with EUK-134 resulted in diminished apical ectodermal ridge cell death as well as parallel reductions in the incidence and severity of limb defects in mouse fetuses (from 67.3% to 35.9%). The forelimb malformations were partially reversed by this manganosalen complex, including postaxial ectrodactyly, metacarpal, and ulnar deficiencies.

4.6. Adrenal and liver diseases

Since the imbalance between free radicals and antioxidants can be suffered by a variety of cells and issues, practically any organ can be affected, leading to a wide variety of pathologies. Kidney and liver function can be altered by excessive ROS and, again, manganosalen complexes appear as antioxidant therapeutic alternatives.

Kregel *et al.* [152] used EUK-189 to prevent age-related oxidative damage associated with environmental stress. They reported that this catalytic antioxidant blocked the activation of activator protein-1 (a redox-sensitive early response transcription factor involved in the regulation of cellular stress responses), enhanced stress tolerance in aged animals by reducing cellular oxidative stress and subsequent accrual of hepatic injury in Fischer 344 rats. Yazdanparast *et al.* [153] reported the amelioration of diet-induced non-alcoholic steatohepatitis in rats by EUK-8 and EUK-134. These two compounds had hepatoprotective, hypolipidemic, hypocholestorolimic and hypoglycemic effects on the *in vivo* model. Thus, the authors reported that EUK-8 and EUK-134 reduced the sera aminotransferases, the extent of lipid peroxidation, low density lipoprotein contents, cholesterol, and protein carbonylation. The same research group published other study about the protective effects of EUK-8, EUK-15, EUK-115, EUK-122, EUK-132 and EUK-134 against CCl_4-induced damages in rats [154]. The manganosalen complexes ameliorated the effects of CCl_4 by decreasing the levels of ROS, lipid and protein oxidations and lipofuscin-like pigments formation on liver and brain.

Peer-reviewed version available at Antioxidants 2020, 9, 727; doi:10.3390/antiox9080727

EUK-134 was used by Ghouleh *et al.* [155] to attenuate the vascular manifestations of sepsis in lipopolysaccharide-treated pigs. This catalytic antioxidant prevented the fall in renal blow flow, an effect associated with a decrease in nitrosative stress in the kidney supporting a renal protective effect.

4.7. Lifespan extension

Harman proposed that organisms age because they accumulate oxidative damage that comes from ROS [156]. His free radical theory of aging prompted investigations to look for therapeutic antioxidants to lifespan extension. Although this theory was supported by later studies that demonstrate that increased production of ROS shortens lifespan [157], and oxidative damage increase with age [158], other reports clearly contradict the basis of this theory [159]. The aim of this review is not to assess this controversy but to present the results of the different tests in this regard.

Table 5. Selected *in vivo* trials of manganosalen complexes treatments for lifespan extension.

Antioxidant model	Animal model	Dose	Outcomes	Ref.
EUK-8 EUK-134	*Caenorhabditis elegans* nematode	0.05 mM	Increase in mean-life span of 44%	1
EUK-8 EUK-134 EUK-189	sod2 nullizygous mice	30 mg/kg	Three-fold lifespan extension	4
EUK-8	*Caenorhabditis elegans* nematode	0.05-5 mM	No increase in lifespan	5
EUK-8 EUK-134	*Caenorhabditis elegans* nematode	0.25-0.5 mM	Increase lifespan in presence of superoxide generators	161
EUK-8 EUK-134	*Caenorhabditis elegans* nematode	0.05-1 mM	Increase lifespan in presence of superoxide generators	162
EUK-8 EUK-134	*Drosophila melanogaster* Fruit fly	0.025-0.5 mM	No extension lifespan in normal animals	163
EUK-8	*Musca domestica* fly	0.025-0.5 mM.	No extension lifespan	164

As already commented in the introductory section Melov *et al.* [1] employed EUK-8 and EUK-134 to increase the mean lifespan of *Caenorhabditis elegans*. On the contrary, Hekimi *et al.* [160] reported that increased oxidative stress caused by deletion of the mitochondrial superoxide dismutase SOD-2 extended lifespan in the same nematode model. According to this latest study, decreased antioxidant function may extend lifespan. *In vivo* studies are sensitive to small changes in the environment, as Gems *et al.* [5] hypothesized to explain their results when they tried to reproduce the lifespan extension of the same nematode using EUK-8. Since they did not find any increase in lifespan upon treatment with this SOD mimetic, they conclude that this effect reported by Melov *et al.* should be very sensitive to subtle differences in the manner in which the manganosalen complex is administered. Their results raised doubts about the potential utility of EUK-8 in the therapeutic attenuation of aging. A subsequent study by Gems *et al.* [161] tested EUK-8 and EUK-134 again in *Caenorhabditis elegans*. In this study, they found that the synthetic mimetics elevated *in vivo* SOD activity levels (increases 5-fold) and exhibited protection when the worms were treated with superoxide generators (paraquat and plumbagin). Thus, the manganosalen complexes increased lifespan in nematodes, compared to the control study, where superoxide levels were elevated but they did not retard aging in the absence of superoxide generators.

EUK-8, EUK-134 and *Caenorhabditis elegans* met again in the study of Lithgow *et al.* [162]. They reported that these manganosalen complexes extended lifespan of the worms and conferred resistance to two types of oxidative stress-inducing agents (paraquat and thermal stress). The protective effects of EUK-8 and EUK-134 were independent of insulin/IGF-I signalling, and they did not show any detrimental repercussion on development or fertility. Definitely, *in vivo* evaluation of these synthetic mimetics in aging studies involves careful consideration of complex concentration, complex delivery and biotic environment.

Partridge *et al.* [163] treated *Drosophila melanogaster*, fruit fly, with EUK-8 and EUK-134, reporting the antioxidant protective effects to rescue pathologies associated with elevated oxidative stress in SOD-deficient flies or normal flies exposed to induced oxidative stress. However, the synthetic antioxidants did not extend lifespan in normal, wild type animals. In a different study, Sohal *et al.* [164], did not find lifespan extension administrating the EUK-8 to *Musca domestica*, both under normoxic and hyperoxic conditions.

In *in vivo* mammalian models, it has already been discussed above how different manganosalen complexes increase the longevity of genetically engineered SOD2 nullizygous mice [4], extending 3-fold their lifespan. In general, the life expectancy of animal models subjected to oxidative stress or with pathologies associated with this stress, is increased with treatment with manganosalen complexes, as reviewed in previous sections. However, the use of these synthetic antioxidants in normal and healthy individuals probably does not have any advantageous effect on lifespan extension.

5. Conclusions

Throughout the previous sections the protective effects of manganosalen complexes to combat oxidative stress and its associated pathologies have been presented and discussed. These compounds showed efficiency to reverse different oxidative damage: neurodegenerative, inflammatory, cardiovascular, adrenal and liver diseases, skin damage and fetal malformations (Figure 10). The increase of lifespan has been also reported for organisms exposed to oxidative stress, although far from being any Elixir of Life. Not all compounds have the same activity, as they show different lipophilicities, redox properties or steric hindrance.

Of the nearly sixty *in vivo* trials, only a few of them did not give beneficial effects for the desired quality. Thus, Wada *et al.* [165] reported that EUK-134 was ineffective in promoting the restoration of prolonged low-frequency force depression, a state which may suffer skeletal muscles under vigorous activity. In this case, the antioxidant catalyst showed a positive effect on sarcoplasmic reticulum Ca^{2+} release in Wistar rats but a negative effect on myofibrillar Ca^{2+} sensitivity. On the other hand, Espósito *et al.* [166] reported the toxicity of EUK-108 (20-100 μM dose) toward *Danio rerio* individuals (zebrafish), particularly in terms of brain damage. Finally, Vanfleteren et al. [11] found that administration of EUK-8 to starving *Escherichia coli* cells surprisingly enhanced the production of ROS, resulting in a massive increase of oxidative damage.

Much is still unknown about the way these manganosalen complexes work in different pathologies. Drugs often have unrecognized effects, so that we cannot be confident that the beneficial effects of these compounds were due solely to their antioxidant activities. More research is needed, particularly because of the properties showed by this type of compounds that also vary with minor structural modifications. Evaluation in animal models continues to be necessary, since it cannot be assumed that the *in vivo* antioxidant activity of different analogues is similar. In the search to identify the best candidate, an orally available one would be the most suitable. Efforts should be directed towards obtaining an effective antioxidant, modulating its lipophilicity and redox potentials, and that it can be administered orally.

Despite the protective effect against ROS shown by these compounds in oxidative stress models *in vitro* and *in vivo*, their practical application in humans remains highly challenge. Clinical trials are crucial to assess the efficacy of this approach, especially after the results given by other antioxidants. Thus, although coenzyme Q10, β-carotene, α-tocopherol or other antioxidant supplements showed highly encouraging results in *in vivo* animal models, most of their clinical trials in humans failed to

reproduce positive results [167-169]. The synthetic catalytic antioxidants approach may have a plus compared with non-enzimatic antioxidants: They may regulate ROS by mimicking the mechanism of the native enzymes.

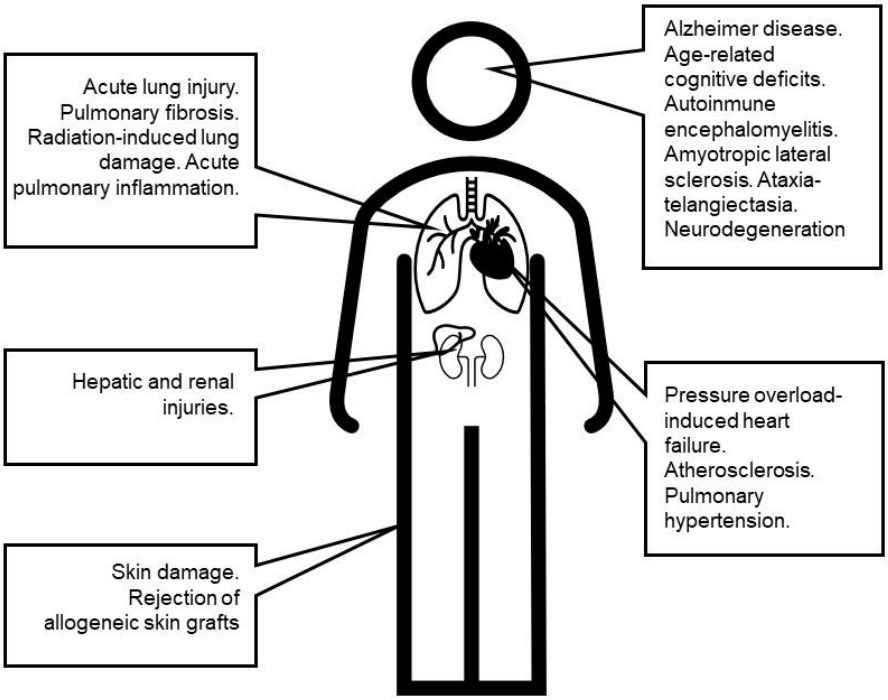

Figure 10. Some of the oxidative stress-related diseases tested in *in vivo* animal models with manganosalen complexes.

Finally, *in vivo* evaluation of pharmacological responses may be affected by multiple factors. Moreover, sometimes the results are simplified by describing them as beneficial or harmful when, turning the sentence of Paracelsus, the dose makes the medicine. ROS play physiological roles in cell signalling and in the control of gene expression, processes that could be affected by antioxidant therapies. The catalytic oxidants should be administered just in the right dose to combat excessive ROS levels.

Author Contributions:

Conceptualization, L. R. and M. M.; methodology, L. R. and M. M.; writing—original draft preparation, , L. R., A. M. G., R. P. and M. M.; writing—review and editing, L. R., A. M. G., R. P. and M. M.; supervision, M. M..; funding acquisition, M. M.

Funding: This research was funded by Xunta de Galicia (GRC GI-1584-ED431C2018/13 Suprabioin Research Group).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Melov, S.; Ravenscroft, J.; Malik, S.; Gill, M. S.; Walker, D. W.; Clayton, P. E.; Wallace, D. C.; Malfroy, B.; Doctrow, S. R.; Lithgow, G. J. Extension of life-span with superoxide dismutase/catalase mimetics. *Science* **2000,** *289*, 1567-1569; DOI: 10.1126/science.289.5484.1567
2. Solomon, L.D. The quest for human longevity: science, business, and public policy, Routledge, New York, 2006.
3. Bracken, M. B. Why animal studies are often poor predictors of human reactions to exposure. *Journal of the Royal Society of Medicine* **2009**, 102, 120-122; DOI: 10.1258/jrsm.2008.08k033
4. Melov, S.; Doctrow, S. R.; Schneider, J. A.; Haberson, J.; Patel, M.; Coskun, P. E.; Huffman, K.; Wallace, D. C.; Malfroy, B. Lifespan extension and rescue of spongiform encephalopathy in superoxide dismutase 2 nullizygous mice treated with superoxide dismutase–catalase mimetics. *Journal of Neuroscience* **2001**, *21*, 8348-8353; DOI: 10.1523/jneurosci.21-21-08348.2001
5. Keaney, M.; Gems, D. No increase in lifespan in Caenorhabditis elegans upon treatment with the superoxide dismutase mimetic EUK-8. *Free Radical Biology and Medicine* **2003**, *34*, 277-282; DOI: 10.1016/s0891-5849(02)01290-x
6. Doctrow, S.R.; Liesa, M.; Melov, S.; Shirihai, O.S.; Tofilon, P. Salen Mn complexes are superoxide dismutase/catalase mimetics that protect the mitochondria.*Current Inorganic Chemistry* **2012**, 2, 325-334 ; DOI : 10.2174/1877944111202030325
7. Kostova, I. ; Sasao, L. Advances in research of Schiff-base metal complexes as potent antioxidants. *Curr. Med. Chem* **2013**, 20, 4609-4632 ; DOI : 10.2174/09298673113209990149
8. Bonetta, R. Potential therapeutic applications of MnSODs and SOD-mimetics. Chem. Eur. J. **2018**, 24, 5032-5041 ; DOI : 10.1002/chem.201704561
9. Kubota, R. ; Asayama, S ; Kawakami, H. Catalytic antioxidants for therapeutic medicine. *J. Mat. Chem. B.* **2019**, 7, 3165-3191; DOI : 10.1039/c8tb03365j
10. Rouco, L. ; Maneiro, M. Neuroprotective effects of metalosalen complexes against oxidative stress. *Neur. Regen. Res*. **2021**, 16, in press.
11. Matthijssens, F.; Back, P.; Braeckman, B. P.; Vanfleteren, J. R. Prooxidant activity of the superoxide dismutase (SOD)-mimetic EUK-8 in proliferating and growth-arrested Escherichia coli cells. *Free Radical Biology and Medicine* **2008**, *45*, 708-715; DOI: 10.1016/j.freeradbiomed.2008.05.023
12. Declercq, L.; Sente, I.; Hellemans, L.; Corstjens, H.; Maes, D. Use of the synthetic superoxide dismutase/catalase mimetic EUK-134 to compensate for seasonal antioxidant deficiency by reducing pre-existing lipid peroxides at the human skin surface. *Int. J. Cosmet. Sci.* **2004**, 26, 255-263; DOI: 10.1111/j.1467-2494.2004.00234.x
13. Batinic-Haberle, I.; Tovmasayan, A.; Roberts, E.R.H.; Vujaskovic, Z.; Leong, K.W.; Spasojevic, I. SOD therapeutics: Latest insights into their structure-activity relationships and impact on the cellular redox-based signaling pathways. *Antioxid. Redox Signal*. **2014**, 20, 2372-2415; DOI: 10.1089/ars.2012.5147
14. Doctrow S.R.; Fish, B.; Huffman, K.D.; Lazarova, Z.; Medhora, M.; Williams, J.P.; Moulder, J.E. Salen manganese complexes mitigate radiation injury in normal tissues through modulation of tissue environment, including through redox mechanisms. *In: Redox-active therapeutics (Oxidative stress in applied basic research and clinical practice)*, 265-285. Springer, Cham.; 2016 DOI: 10.1007/978-3-319-30705-3_11
15. Walke, G. R.; Ranade, D. S.; Bapat, A. M.; Srikanth, R.; Kulkarni, P. P. Mn(III)-salen protect against different ROS species generated by the Aβ16-Cu complex. *ChemistrySelect* **2016**, 1, 3497-3501; DOI: 10.1002/slct.201600808
16. Baglia, B. A.; Zaragoza, J.P.T.; Goldberg. Biomimetic reactivity of oxygen-derived manganese and iron porphyrinoid complexes. *Chem. Rev.* **2017**, 117, 13320-13352; DOI: 10.1021/acs.chemrev.7b00180
17. Rice, D. B.; Massie, A. A.; Jackson, T. A. Manganese-oxygen intermediates in O-O bond activation and hydrogen-atom transfer reactions. *Acc. Chem. Res*. **2017**, 50, 2706-2717; DOI: 10.1021/acs.accounts.7b00343
18. Yin, H.Y.; Tang, J.; Zhang, J.L. Introducing metallosalens into biological studies: the renaissance of traditional coordination complexes. *Eur. J. Inorg. Chem.* **2017**, 44, 5085-5093; DOI: 10.1002/ejic.201700695
19. Liberato, A.; Fernández-Trujillo, M.J.; Máñez, A.; Maneiro, M.; Rodríguez-Silva, L.; Basallote, M.G. Pitfalls in the ABTS peroxidase activity test: Interference of photochemical processes. *Inorg. Chem.* **2018**, 57,14471-14475; DOI: 10.1021/acs.inorgchem.8b02525

20. Signorella, S.; Palopoli, C.; Ledesma, G. Rationally designed mimics of antioxidant manganoenzymes: Role of structural features in the quest for catalysts with catalase and superoxide dismutase activity. *Coord. Chem. Rev.* **2018**, 305, 75-102; DOI: 10.1016/j.ccr.2018.03.005
21. Erxleben, A. Transition metal salen complexes in bioinorganic and medicinal chemistry. *Inorg. Chim. Acta* **2018**, 472, 40-57; DOI: 10.1016/j.ica.2017.06.060
22. Benkafadar, N.; François, F.; Affortit, C.; Casas, F.; Ceccato, J.C.; Menardo, J.; Venail, F.; Malfroy-Camine, B.; Puel, J.L.; Wang, J. ROS-induced activation of DNA damage responses drives senescence-like state in postmitotic cochlear cells: Implication for hearing preservation. *Mol. Neurobiol.* **2019**, 56, 5950-5969; DOI: 10.1007/s12035-019-1493-6
23. Rouco, L.; Liberato, A.; Fernández-Trujillo, M.J.; Máñez, A.; Basallote, M.G.; Alvariño, R.; Alfonso, A.; Botana, L.M.; Maneiro, M. Salen-manganese complexes for controlling ROS damage: Neuroprotective effects, antioxidant activity and kinetic studies. *J. Inorg. Biochem.* **2020**, 203, 110918; DOI: 10.1016/j.jinorgbio.2019.110918
24. Villamena, F. A. Chemistry of Reactive Species. *Molecular Basis of Oxidative* **2013**, 1-48 ; DOI : 10.1002/9781118355886.ch1
25. Lakey, P. S. J. ; Berkemeier, T. ; Tong, H. ; Arangio, A. M. ; Lucas, L. ; Pöschl, U. ; Shiraiwa, M. Chemical exposure-response relationship between air pollutants and reactive oxygen species in the human respiratory tract. *Sci. Rep.* **2016**, 6, 32916 ; DOI : 10.1038/srep32916
26. Church, D.F.; Pryor, W.A. Free-radical chemistry of cigarette smoke and its toxicological implications. *Environ Health Perspect* **1985**, 64, 111–126; DOI: 10.1289/ehp.8564111
27. Narayanan, P.K.; Goodwin, E.H.; Lehnert, B.E. Alpha particles initiate biological production of superoxide anions and hydrogen peroxide in human cells. *Cancer Res.* **1997**, 57, 3963–3971.
28. Stohs, S.J.; Bagchi, D. Oxidative mechanisms in the toxicity of metal ions. *Free Radic Biol Med.* **1995**, 18, 321–336; DOI: 10.1016/0891-5849(94)00159-h
29. Ristow, M.; Schmeisser, S. Extending life span by increasing oxidative stress. *Free Radic. Biol. Med.* **2011**, 51, 327–336; DOI : 10.1016/j.freeradbiomed.2011.05.010
30. Zarse, K.; Schmeisser, S.; Groth, M.; Priebe, S.; Beuster, G.; Kuhlow, D.; Guthke, R.; Platzer, M.; Kahn, C.R.; Ristow, M. Impaired insulin/IGF1 signaling extends life span by promoting mitochondrial L-proline catabolism to induce a transient ROS signal. *Cell Metab*. **2012**, 15, 451–465; DOI : 10.1016/j.cmet.2012.02.013
31. Nyska, A. ; Kohen, R. Oxidation of biological systems : Oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicol. Pathol.* **2002**, 30, 620-650 ; DOI : 10.1080/01926230290166724
32. Stadtman, E. R. ; Levine, R. L. Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. *Amino acids* **2003**, 25, 207-218; DOI : 10.1007/s00726-003-0011-2
33. Lin, M.T.; Beal M.F. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature* **2006**, 443, 787–795; DOI: 10.1038/nature05292
34. Ott, M.; Gogvadze, V.; Orrenius, S.; Zhivotovosky, B. Mitochondria, oxidative stress and cell death. *Apoptosis* **2007**, 12, 913-922; DOI: 10.1007/s10495-007-0756-2
35. Halliwell, B.; Gutteridge J.M.C. *Free Radicals in Biology and Medicine*, Oxford University Press, USA, 2007; p.p. 79-186.
36. Cerutti, P. A. Oxidant stress and carcinogenesis. *Eur. J. Clin. Invest.* **1991**, 21, 1-5; DOI: 10.1111/j.1365-2362.1991.tb01350.x
37. Valko, M.; Leibfritz, D.; Moncol, J.; Cronin, M. T. D.; Mazur, M.; Tesler, J. Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol.* **2007**, 39, 44-84; DOI: 10.1016/j.biocel.2006.07.001
38. Halliwell, B. Antioxidants and human disease: a general introduction. *Nutr. Rev.* **1997**, 55, S44-S49; DOI: 10.1111/j.1753-4887.1997.tb06100.x
39. Seedek, M.; Callera, G.; Montezano, A.; Gutsol, A.; Heitz, F.; Szyndralewiez, C.; Page, P.; Kennedy, C. R. J.; Burns, K. D.; Touyz, R. M.; Hebert, R. L. Critical role of Nox-4 based NADPH oxidase in glucose-induced oxidative stress in the kidney: implications in type 2 diabetic nephropathy. *Am J. Physiol. Renal Physiol.* **2010**, 299, F1348-F1358; DOI: 10.1152/ajprenal.00028.2010
40. Chandrasekaran, A.; Idelchik, M. D. S.; Melendez, J. A. Redox control of senescence and age-related disease. *Redox Biol.* **2017**,11, 91-102; DOI: 10.1016/j.redox.2016.11.005

41. Zhao, X.Y.; Lu, M.H.; Yuan, D.J.; Xu, D.E.; Yao, P.P.; Ji, W.L.; Chen, H.; Liu, W.L.; Yan, C.X.; Xia, Y.Y.; Li, S.; Tao, J.; Ma, Q.H. Mitochondrial dysfunction in neural injury. *Front. Neurosci*. **2019**, 13, 30; DOI: 10.3389/fnins.2019.00030
42. Sharma, P.; Srivastava, P.; Seth, A.; Tripathi, P.N.; Banerjee, A.G.; Shrivastava, S.K. Comprehensive review of mechanisms of pathogenesis involved in Alzheimer's disease and potential therapeutic strategies. *Progress in Neurobiology* **2019**, 174, 53-89; DOI: 10.1016/j.pneurobio.2018.12.006
43. He, L.; He, T.; Farrar, S.; Ji, L. B.; Liu, T. Y.; Ma, X. Antioxidants maintain cellular redox homeostasis by elimination of reactive oxygen species. *Cell Physiol. Biochem.* **2017**, 44, 532-553; DOI: 10.1159/000485089
44. McCord, J. M.; Fridovich, I. Superoxide dismutase an enzymic function for erythrocuprein (hemocuprein). *J. Biol. Chem.***1969**, 244, 6049–6055; DOI: 10.1074/jbc.m007891200
45. Perry, J. J. P.; Shin, D. S.; Getzoff, E. D.; Tainer, J. A. The structural biochemistry of the superoxide dismutases. *Biochim. Biophys. Acta* **2010**, 1804, 245-262; DOI: 10.1016/j.bbapap.2009.11.004
46. Azadmanesh, J. ; Borgstahl, G.E.O. A review of the catalytic mechanism of human manganese superoxide dismutase. *Antioxidants* **2018**, 7, 25; DOI : 10.3390/antiox7020025
47. Sheng, Y.; Abreu, I. A.; Cabelli, D. E.; Maroney, M. J.; Miller, A. F.; Teixeira, M.; Valentine, J. S. Superoxide dismutases and superoxide reductases. *Chem. Rev.* **2014**, 114, 3854-3918; DOI: 10.1021/cr4005296
48. Oszajca, M.; Brindell, M.; Orzeł, Ł.; Dąbrowski, J. M.; Śpiewak, K.; Łabuz, P.; Stochel, G. Mechanistic studies on versatile metal-assisted hydrogen peroxide activation processes for biomedical and environmetal incentives. *Coord. Chem. Rev.* **2016**, 143, 327-328; DOI: 10.1016/j.ccr.2016.05.013
49. Whittaker, J. W. Non-heme manganese catalase–the 'other'catalase. *Arch. Biochem. Biophys.* **2012**, 525, 111–120; DOI: 10.1016/j.abb.2011.12.008
50. Barynin, V. V.; Whittaker, M. M.; Antonyuk, S. V.; Lamzin, V. S.; Harrison, P. M.; Artymiuk, P. J.; Whittaker, J. W. Crystal structure of manganese catalase from Lactobacillus Plantarum. *Structure* **2001**, 9, 725-738; DOI: 10.1016/s0969-2126(01)00628-1
51. Frausto da Silva, J. J. R.; Williams, R. J. P. *The biological chemistry of the elements: the inorganic chemistry of life*. Oxford University Press, 2001.
52. Sundaramoorthy, M. ; Gold, M. H. ; Poulos, T. L. Ultrahigh (0.93 Å) resolution structure of manganese peroxidase from Phanerochaete chrysosporium: Implications for the catalytic mechanism. *J. Inorg Biochem.* **2010**, 104, 683-690; DOI: 10.1016/j.jinorgbio.2010.02.011
53. Imai, H.; Nakagawa. Biological significance of phospholipid hydroperoxide glutathione peroxidase (PHGPx, GPx4) in mammalian cells. *Free Radic. Biol. Med.* **2003**, 34, 145-169; DOI: 10.1016/s0891-5849(02)01197-8
54. Bilal, M.; Rasheed, T.; Iqbal, H. M. N.; Yan, Y. J. Peroxidases-assisted removal of environmentally-related hazardous pollutants with reference to the reaction mechanisms of industrial dyes. *Sci. Total Environ.* **2018**, 644, 1-13; DOI: 10.1016/j.scitotenv.2018.06.274
55. Giustarini, D.; Dalle-Donne, I.; Tsikas, D.; Rossi, R. Oxidative stress and human diseases: origin, link, measurement, mechanisms, and biomarkers. *Critical reviews in clinical laboratory sciences* **2009**, 46, 241-281; DOI: 10.3109/10408360903142326
56. Davalli, P.; Mitic, T.; Caporali, A.; Lauriola, A.; D'Arca, D. ROS, cell senescence, and novel molecular mechanisms in aging and age-related diseases. *Oxidative medicine and cellular longevity* **2016**; DOI: 10.1016/j.addr.2009.02.005
57. Fang, J.; Seki, T.; Hiroshi, M. Therapeutic strategies by modulating oxygen stress in cancer and inflammation. *Adv. Drug Deliv. Rev.* **2009**, 61, 290-302; DOI: 10.1016/j.addr.2009.02.005.
58. Venigalla, M.; Sonego, S.; Sharman, M. J.; Much. G. Novel promising therapeutics against chronic neuroinflammation and neurodegeneration in Alzheimer's disease. *Neurchem. Int.* **2016**, 95, 63-74; DOI: 10.1016/j.neuint.2015.10.011.
59. Essa, M. M.; Moghadas, M.; Ba-Omar, T.; Qoronfleh, M. W.; Guillemin, G. J.; Manivasagam, T.; Justin-Thenmozhi, A.; Ray, R.; Bhat, A.; Chidambaram, S. B.; Fernandes, A. J.; Song, B. J.; Akbar, M. Protective effects of antioxidants in Huntington's disease: an extensive review. *Neurotox. Res.* **2019**, 3, 739-774; DOI: 10.1007/s12640-018-9989-9.
60. Teodoro, J. S.; Nunes, S.; Rolo, A. P.; Reis, F.; Palmeira, C. M. Therapeutic options targeting oxidative stress, mitochondrial dysfunction and inflammation to hinder the progression of vascular complications of diabetes. *Front Physiol.* **2019**, 9, 1857; DOI: 10.3389/fphys.2018.01857.

61. Kurutas, E. B. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutr. J.* **2016**, 15, 71; DOI: 10.1186/s12937-016-0186-5.
62. Propac, P.; Jomova, K.; Simunkova, M.; Kollar, V.; Rhodes, C. J.; Valko, M. Targeting free radicals in oxidative stress-related human diseases. *Trends Pharmacol. Sci.* **2017**, 38, 592-607; DOI: 10.1016/j.tips.2017.04.005.
63. Rosenthal, R. A.; Huffman, K. D.; Fisette, L. W.; Damphousse, C. A.; Callaway, W. B.; Malfroy, B.; Doctrow, S. Orally available Mn porphyrins with superoxide dismutase and catalase activities. *J. Biol. Inorg. Chem.* **2009**, 14, 979-991; DOI: 10.1007/s00775-009-0550-4
64. Coudriet, G.M.; Delmastro-Greenwood, M.M.; Previte, D.M.; Marre, M.L.; O'Connor, E.C.; Novak, E.A.; Vincent, G.; Mollen, K.P.; Lee, S.; Dong, H.H.; et al. Treatment with a Catalytic Superoxide Dismutase (SOD) Mimetic Improves Liver Steatosis, Insulin Sensitivity, and Inflammation in Obesity-Induced Type 2 Diabetes. *Antioxidants* **2017**, 6, 85; DOI: 10.3390/antiox6040085
65. Shrishrimal, S.; Kosmacek, E.A.; Chatterjee, A.; Tyson, M.J.; Oberley-Deegan, R.E. The SOD Mimic, Mnte-2-PyP, Protects from Chronic Fibrosis and Inflammation in Irradiated Normal Pelvic Tissues. *Antioxidants* **2017**, 6, 87; DOI: 10.3390/antiox6040087
66. Galli, F.; Azzi, A.; Birringer, M.; Cook-Mills, J. M.; Eggersdorfer, M.; Frank, J.; Cruciani, G.; Lorkowski, S.; Ozer, N. K. Vitamin E: emerging aspects and new directions. *Free Rad. Biol. Med.* **2017**, 102, 16-36; DOI: 10.1016/j.freeradbiomed.2016.09.017.
67. Padayatty, S. J.; Katz, A.; Wang, Y. H.; Eck, P.; Kwon, O.; Lee, J. H.; Chen, S. L.; Corpe, C.; Dutta, A.; Dutta, S. K.; Levine, M. Vitamin C as an antioxidant: evaluation of its role in disease prevention. *J. Am. Coll. Nutr.* **2003**, 22, 18-35; DOI: 10.1080/07315724.2003.10719272.
68. Fiedor, J.; Burda, K. Potential role of carotenoids as antioxidants in human health and disease. *Nutrients* **2014**,6, 466-488; DOI: 10.3390/nu6020466.
69. Milani, A.; Basirnejad, M.; Shahbazi, S.; Bolhassani, A. Carotenoids: biochemistry, pharmacology and treatment. *Br. J. Pharmacol*. **2017**,174, 1290-1324; DOI: 10.1111/bph.13625.
70. Cominacini, L.; Fratta Pasini, A.; Garbin, U.; Pastorino, A. M.; Davoli, A.; Nava C.; Campagnola, M.; Rossato, P.; Lo Cascio, V. Antioxidant activity of different dihydropyridines. *Biochem. Biophys. Res. Commun.* **2003**, 302, 679-684; DOI: 10.1016/S0006-291X(03)00158-X.
71. Pei, K. H.; Ou, J. Y.; Huang, J. Q.; Ou, S. Y. p-coumaric acid and its conjugates: dietary sources, pharmacokinetic properties and biological activities. *J. Sci. Food Agric.* **2016**, 96, 2952-2962; DOI: 10.1002/jsfa.7578.
72. Peluso, I.; Serafini, M. Antioxidants from black and green tea: from dietary modulation of oxidative stress to pharmacological mechanisms. *Br. J. Pharmacol.* **2017**,174, 1195-1208; DOI: 10.1111/bph.13649.
73. Ginwala, R.; Bhavsar, R.; Chigbu, D. I.; Jain, P.; Khan, Z. K. Potential role of flavonoids in treating chronic inflammatory diseases with a special focus on the anti-inflammatory activity of apigenin. *Antioxidants* **2019**, 8, 35; DOI: 10.3390/antiox8020035.
74. Pfeiffer, P.; Breith, E.; Lübbe, E.; Tsumaki, T. Tricyclische orthokondensierte nebenvalenzringe. *Justus Liebigs Ann. Chem.* **1933**, 503, 84-130; DOI: 10.1002/jlac.19335030106.
75. Katsuki, T. Some recent advances in metallosalen chemistry. *Synlett* **2003**, 3, 281-297; DOI: 10.1055/s-2003-37101.
76. Canali, L.; Sherrington, D. C. Utilisation of homogeneous and supported chiral metal(salen) complexes in asymmetric catalysis. *Chem. Soc. Rev.* **1999**, 28, 85-93; DOI: 10.1039/a806483k.
77. Shaw, S.; White, J. D. Asymmetric catalysis using chiral salen-metal complexes: recent advances. *Chem. Rev.* **2019**, 119, 9381-9426; DOI: 10.1021/acs.chemrev.9b00074.
78. Baleizao, C.; Garcia, H. Chiral salen complexes: an overview to recoverable and reusable homogeneous and heterogeneous catalysts. *Chem. Rev.* **2006**, 106, 3987-4043; DOI: 10.1021/cr050973n.
79. Pessoa, J. C.; Correia, I. Salan vs. salen metal complexes in catalysis and medicinal applications: Virtues and pitfalls. *Coord. Chem. Rev.* **2019**, 388, 227-247; DOI: 10.1016/j.ccr.2019.02.035.
80. Chandra, P.; Ghosh, T.; Choudhary, N.; Mohammad, A.; Mobin, S. M. Recent advancement in oxidation or acceptorless dehytdrogenation of alcohols to valorised products using manganese based catalysts. *Coord. Chem. Rev.* **2020**, 411, 213241; DOI: 10.1016/j.ccr.2020.213241.
81. Vázquez-Fernández, M. Á.; Bermejo, M. R.; Fernández-García, M. I.; González-Riopedre, G.; Rodríguez-Doutón, M. J.; Maneiro, M. Influence of the geometry around the manganese ion on the peroxidase and

catalase activities of Mn(III)-Schiff base complexes. *J. Inorg. Biochem.* **2011**, **105,** 1538-1547; DOI: 10.1016/j.jinorgbio.2011.09.002.

82. Vázquez-Fernández, M. A.; Fernández-García, M. I.; González-Riopedre, G.; Maneiro, M.; Rodríguez-Doutón, M. J. Self-assembled biomimetic catalysts: studies of the catalase and peroxidase activities of Mn(III)-Schiff base complexes. *J. Coord. Chem.* **2011**, 64, 3843-3858; DOI: 10.1080/00958972.2011.633164.
83. González-Riopedre, G.; Bermejo, M. R.; Fernández-García, M. I.; González-Noya, A. M.; Pedrido, R.; Rodríguez-Doutón, M.; Maneiro, M. Alkali-metal-ion-directed self-assembly of redox-active manganese(III) supramolecular boxes. *Inorg. Chem.* **2015**, 54, 2512-2521; DOI: 10.1021/ic501842x.
84. Bermejo, M. R.; Carballido, R.; Fernandez-García, M. I.; González-Noya, A. M.; González-Riopedre, G.; Maneiro, M.; Rodríguez-Silva, L. Synthesis, characterization, and catalytic studies of Mn(III)-Schiff base-dicyanamide complexes: Checking the rhombicity effect in peroxidase studies. *J. Chem.* **2017**, 5465890; DOI: 10.1155/2017/5465890.
85. Doctrow, S. R.; Huffman, K.; Bucay Marcus C.; Tocco, G.; Malfroy, E.; Adinolfi, C. A.; Kruk, H.; Baker, K.; Lazarowych, N.; Mascarenhas, J.; Malfroy, B. Salen-manganese complexes as catalytic scavengers of hydrogen peroxide and cytoprotective agents: structure-activity relationship studies. *J. Med. Chem.* **2002**, 45, 4549-4558; DOI: 10.1021/jm020207y.
86. Vázquez-Fernández, M. Á.; Fernández-García, M. I.; González-Noya, A. M.; Maneiro, M.; Bermejo, M. R.; Rodríguez-Doutón, M. J. Supramolecular networks of Mn(III)-Schiff base complexes assembled by nitrate counterions: X-ray crystal structures of 1D chains and 2D networks. *Polyhedron* **2012**, 31, 379-385; DOI: 10.1016/j.poly.2011.09.031
87. Palopoli, C.; Ferreyra, J.; Conte-Daban, A.; Richezzi, M.; Foi, A.; Doctorovich, F.; Anxolabehere-Mallart, E.; Hureau, C.; Signorella, S. R. Insights into second-sphere effects on redox potentials, spectroscopic properties and superoxide dismutase activity of manganese complexes with Schiff-base ligands. *ACS Omega* **2019**, 4, 48-57; DOI: 10.1021/acsomega.8b03018.
88. Ansari, K. I.; Kasiri, S.; Grant, J. D.; Mandal, S. S. Apoptosis and anti-tumour activities of manganese(III)-salen and -salphen complexes. *Dalton Trans.* **2009**, 40, 8525-8531; DOI: 10.1039/b905276c.
89. Bahramikia, S.; Yazdanparast, R.; Gheysarzadeh, A. Syntheses and structure-activity relationships of seven manganese-salen derivatives as anti-amyloidogenic and fibril-destabilizing agents against hen egg-white lysozyme aggregation. *Chem. Biol. Drug Des.* **2012**, 80, 227-236; DOI: 10.1111/j.1747-0285.2012.01391.x.
90. Signorella, S.; Hureau, C. Bioinspired functional mimics of the manganese catalases. *Coord. Chem. Rev.* **2012**, 256, 1229-1245; DOI: 10.1016/j.ccr.2012.02.003.
91. Kinnula, V. L.; Crapo, J. D. Superoxide dismutases in the lung and human lung diseases. *Am. J. Respir. Crit. Care Med*. **2003**, 167, 1600–1619; DOI: 10.1164/rccm.200212-1479so
92. Watanabe, T.; Owada, S.; Kobayashi, H. P.; Kawakami, H.; Nagaoka, S.; Murakami, E.; Tobe, N.; et al. Protective effects of MnM2Py4P and Mn-salen against small bowel ischemia/reperfusion injury in rats using an in vivo and an ex vivo electron paramagnetic resonance technique with a spin probe. *Transplant. Proc.* **2007**, 39, 3002–3006; DOI: 10.1016/j.transproceed.2007.08.091
93. Baudry, M.; Etienne, S.; Bruce, A.; Palucki, M.; Jacobsen, E.; Malfroy, B. Salen-manganese complexes are superoxide dismutase-mimics. Biochem. Biophys. *Res. Commun.* **1993**, 192, 964-968; DOI: 10.1006/bbrc.1993.1509.
94. Friedel, F. C.; Lieb, D.; Ivanovic-Burmazovic, I. Compartive studies on manganese-based SOD mimetics, including the phosphate effect, by using global spectral analysis. *J. Inorg. Biochem.* **2012**, 109, 26-32; DOI: 10.1016/j.jinorgbio.2011.12.008.
95. Batinić-Haberle, I.; Rebouças, J. S.; Spasojević, I. Superoxide dismutase mimics: chemistry, pharmacology, and therapeutic potential. *Antioxid. Redox Signaling* **2010**, 13, 877–918; DOI: 10.1089/ars.2009.2876
96. Day, B. J. Catalase and glutathione peroxidase mimics. *Biochem. Parmacol.* **2009**, 77, 285-296; DOI: 10.1016/j.bcp.2008.09.029.
97. Gonzalez, P. K.; Zhuang, J.; Doctrow, S. R.; Malfroy, B.; Smith, M.; Menconi, M. J.; Fink, M. P. Delayed treatment with EUK-8, a novel synthetic superoxide dismutase (SOD) and catalase (CAT) mimetic, ameliorates acute lung injury in endotoxemic pigs. *Surg. Forum* **1995**, *46*, 72-73.
98. Watanabe, Y.; Namba, A.; Umezawa, N.; Kawahata, M.; Yamaguchi, K.; Higuchi, T. Enhanced catalase-like activity of manganese salen complexes in water: effect of a three-dimensionally fixed auxiliary. *Chem. Commun.* **2006**, 47, 4958-4960; DOI: 10.1039/b608846e

99. Yang, J. I.; Nocera, D. G. Catalase and epoxidation activity of manganese salen complexes bearing two xanthene scaffolds. *J. Am. Chem. Soc.* **2007**, 129, 8192-8198; DOI: 10.1021/ja070358w
100. Maneiro, M.; Bermejo, M. R.; Fernández, M. I.; Gómez-Fórneas, E.; González-Noya, A. M.; Tyryshkin, A. M. A new type of manganese-Schiff base complex, catalysts for the disproportionation of hydrogen peroxide as peroxidase mimics. *New J. Chem.* **2003**, 27, 727-733; DOI: 10.1039/b210738d.
101. Doctrow, S. R.; Huffman, K.; Marcus, C. B.; Musleh, W.; Bruce, A.; Baudry, M.; Malfroy, B. Salen-manganese complexes: combined superoxide dismutase/catalase mimics with broad pharmacological efficacy. Adv Pharmacol **1996**, *38*, 247–269; DOI: 10.1016/s1054-3589(08)60987-4
102. Rong, Y.; Doctrow, S.R.; Tocco, G.; Baudry, M. EUK-134, a synthetic superoxide dismutase and catalase mimetic, prevents oxidative stress and attenuates kainate-induced neuropathology. Proc Natl Acad Sci **1999**, *96*, 9897–9902; DOI: 10.1073/pnas.96.17.9897
103. Malfroy, B.; Doctrow, S.R.; Orr, P.L.; Tocco, G.; Fedoseyeva, E.V.; Benichou, G. Prevention and suppression of autoimmune encephalomyelitis by EUK-8, a synthetic catalytic scavenger of oxygen-reactive metabolites. Cell Immunol **1997**, *177*, 62–68; DOI: 10.1006/cimm.1997.1091
104. Hinerfeld, D.; Traini, M. D.; Weinberger, R. P.; Cochran, B.; Doctrow, S. R.; Harry, J.; Melov, S. Endogenous mitochondrial oxidative stress: neurodegeneration, proteomic analysis, specific respiratory chain defects, and efficacious antioxidant therapy in superoxide dismutase 2 null mice. *Journal of neurochemistry* **2004**, *88*, 657-667; DOI: 10.1046/j.1471-4159.2003.02195.x.
105. Melov, S.; Wolf, N.; Strozyk, D.; Doctrow, S. R.; Bush, A. I. Mice transgenic for Alzheimer disease β-amyloid develop lens cataracts that are rescued by antioxidant treatment. *Free Radic. Biol. Med*. **2005**, *38*, 258-261; DOI: 10.1016/j.freeradbiomed.2004.10.023.
106. Forster, M. J.; Dubey, A.; Dawson, K. M.; Stutts, W. A.; Lal, H.; Sohal, R. S. Age-related losses of cognitive function and motor skills in mice are associated with oxidative protein damage in the brain. Proc. Natl. Acad. Sci. USA **1996**, *93*, 4765-4769, DOI: 10.1073/pnas.93.10.4765.
107. Liu, R.; Liu, I. Y.; Bi, X.; Thompson, R. F.; Doctrow, S. R.; Malfroy, B.; Baudry, M. Reversal of age-related learning deficits and brain oxidative stress in mice with superoxide dismutase/catalase mimetics. Proc. Natl. Acad. Sci. **2003**, *100*, 8526-8531; DOI: 10.1073/pnas.1332809100.
108. Clausen, A.; Doctrow, S.; Baudry, M. Prevention of cognitive deficits and brain oxidative stress with superoxide dismutase/catalase mimetics in aged mice. Neurobiology of aging **2010**, *31*, 425-433; DOI: 10.1016/j.neurobiolaging.2008.05.009.
109. Malfroy, B.; Doctrow, S. R.; Orr, P. L.; Tocco, G.; Fedoseyeva, E. V.; Benichou, G. Prevention and suppression of autoimmune encephalomyelitis by EUK-8, a synthetic catalytic scavenger of oxygen-reactive metabolites. *Cell. immunol*. **1997**, *177*, 62-68; DOI: 10.1006/cimm.1997.1091.
110. Watanabe, T.; Owada, S.; Kobayashi, H. P.; Kawakami, H.; Nagaoka, S.; Murakami, E.; Tobe, N. Protective effects of MnM2Py4P and Mn-salen against small bowel ischemia/reperfusion injury in rats using an in vivo and an ex vivo electron paramagnetic resonance technique with a spin probe. *Transplant. Proc.* **2007,** *39*, 3002-3006; DOI: 10.1016/j.transproceed.2007.08.091.
111. Batinic-Haberle, I.; Benov, L.; Spasojevic, I.; Fridovich, I. The ortho effect makes manganese (III) meso-tetrakis (N-methylpyridinium-2-yl) porphyrin a powerful and potentially useful superoxide dismutase mimic. *J. Biol. Chem.* **1998**, *273*, 24521-24528; DOI: 10.1074/jbc.273.38.24521
112. Jung, C.; Rong, Y.; Doctrow, S.; Baudry, M.; Malfroy, B.; Xu, Z. Synthetic superoxide dismutase/catalase mimetics reduce oxidative stress and prolong survival in a mouse amyotrophic lateral sclerosis model. *Neurosci. Lett.* **2001**, *304*, 157-160; DOI: 10.1016/S0304-3940(01)01784-0.
113. Baker, K.; Marcus, C.B.; Huffman, K.; Kruk, H.; Malfroy, B.; Doctrow. S.R. Synthetic combined superoxide dismutase/catalase mimetics are protective as a delayed treatment in a rat stroke model: a key role for reactive oxygen species in ischemic brain injury. *J. Pharmacol. Exp. Ther.* **1998**, *284*, 215-221.
114. Peng, J.; Stevenson, F.F.; Doctrow, S.R.; Andersen, J.K. Superoxide Dismutase/Catalase Mimetics Are Neuroprotective against Selective Paraquat-mediated Dopaminergic Neuron Death in the Substantial Nigra. *J. Biol. Chem.* **2005**, *280*, 29194-29198; DOI: 10.1074/jbc.M500984200.
115. Brazier, M.W.; Doctrow, S.R.; Masters, C.L.; Collins, S.J. A manganese-superoxide dismutase/catalase mimetic extends survival in a mouse model of human prion disease. *Free Radic. Biol. Med.* **2008**, *45*, 184-192; DOI: 10.1016/j.freeradbiomed.2008.04.006.

116. Browne, S.E.; Roberts II, L.J.; Dennery, P.A.; Doctrow, S.R.; Beal, M.F.; Barlow, C.; Levine, R.L. Treatment with a catalytic antioxidant corrects the neurobehavioral defect in ataxia-telangiectasia mice. *Free Radic. Biol. Med.* **2004**, *36*, 938-942; DOI: 10.1016/j.freeradbiomed.2004.01.003.
117. Raber, J.; Davis, M. J.; Pfankuch, T.; Rosenthal, R.; Doctrow, S. R.; Moulder, J. E. Mitigating effect of EUK-207 on radiation-induced cognitive impairments. *Behav. Brain Res.* **2017,** *320*, 457-463; DOI: 10.1016/j.bbr.2016.10.038.
118. Wang, J.; Puel, J. L. Presbycusis: An Update on Cochlear Mechanisms and Therapies. *J. Clinic. Med.* ***2020***, *9*, 218; DOI: 10.3390/jcm9010218.
119. Burgering, B.M.; Medema, R.H. Decisions on life and death: FOXO Forkhead transcription factors are in command when PKB/Akt is off duty. *J. Leukoc. Biol.* **2003**, *73*, 689–701; DOI: 10.1189/jlb.1202629.
120. Zhang, X.; Chen, X.; Song, H.; Chen, H.Z.; Rovin, B.H. Activation of the Nrf2/antioxidant response pathway increases IL-8 expression. *Eur. J. Immunol.* **2005**, *35*, 3258–3267; DOI: 10.1002/eji.200526116.
121. Muller, J.M.; Rupec, R.A.; Baeuerle, P.A. Study of gene regulation by NF-kappa B and AP-1 in response to reactive oxygen intermediates. *Methods* **1997**, *11*, 301–312; DOI: 10.1006/meth.1996.0424
122. Langan, A. R.; Khan, M. A.; Yeung, I. W.; Van Dyk, J.; Hill, R. P. Partial volume rat lung irradiation: the protective/mitigating effects of Eukarion-189, a superoxide dismutase–catalase mimetic. *Radiother. oncol.* **2006**, *79*, 231-238; DOI: 10.1016/j.radonc.2006.03.017.
123. Kim, J. H.; Lawler, J. M. Amplification of proinflammatory phenotype, damage, and weakness by oxidative stress in the diaphragm muscle of mdx mice. *Free Radic. Biol. Med*. **2012,** *52*, 1597-1606; DOI: 10.1016/j.freeradbiomed.2012.01.015.
124. Howard, M. D.; Greineder, C. F.; Hood, E. D.; Muzykantov, V. R. Endothelial targeting of liposomes encapsulating SOD/catalase mimetic EUK-134 alleviates acute pulmonary inflammation. *J. Control. Release* **2014,** *177*, 34-41; DOI: 10.1016/j.jconrel.2013.12.035.
125. Kumar, A.; Shukla, S.; Chauhan, A. K.; Singh, D.; Pandey, H. P.; Singh, C. The manganese-salen compound EUK-134 and N-acetyl cysteine rescue from zinc-and paraquat-induced toxicity in rat polymorphonuclear leukocytes. *Chem.-biol. interact*. **2015**, *231*, 18-26; DOI: 10.1016/j.cbi.2015.02.012.
126. Mahmood, J.; Jelveh, S.; Zaidi, A.; Doctrow, S. R.; Hill, R. P. Mitigation of radiation-induced lung injury with EUK-207 and genistein: effects in adolescent rats. *Radiat. Res.* **2013,** *179*, 125-134; DOI: 10.1667/RR2954.1.
127. Kash, J. C.; Xiao, Y.; Davis, A. S.; Walters, K. A.; Chertow, D. S.; Easterbrook, J. D.; Dunfee, R. L.; Sandouk, A.; Jagger, B. W.; Schwartzman, L. M.; Kuestner, R. E.; Wehr, N. B.; Huffman, K.; Rosenthal, R. A.; Ozinsky, A.; Levine, R. L.; Doctrow, S. R.; Taubenberger, J. K. Treatment with the reactive oxygen species scavenger EUK-207 reduces lung damage and increases survival during 1918 influenza virus infection in mice. *Free Radic. Biol. Med*. **2014,** *67*, 235-247; DOI: 10.1016/j.freeradbiomed.2013.10.014.
128. Kobasa, D.; Jones, S.M.; Shinya, K.; Kash, J.C.; Copps, J.; Ebihara, H.; Hatta, Y.; Kim, J.H.; Halfmann, P.; Hatta, M.; Feldmann, F.; Alimonti, J.B.; Fernando, L.; Li, Y.; Katze, M.G.; Feldmann, H.; Kawaoka, Y. Aberrant innate immune response in lethal infection of macaques with the 1918 influenza virus. *Nature* **2007**, *445*, 319-323; DOI: 10.1038/nature05495.
129. Gonzalez, P. K.; Zhuang, J.; Doctrow, S. R.; Malfroy, B.; Benson, P. F.; Menconi, M. J.; Fink, M. P. EUK-8, a synthetic superoxide dismutase and catalase mimetic, ameliorates acute lung injury in endotoxemic swine. *J. Pharmacol. Exp. Ther.* **1995**, *275*, 798-806; DOI: 10.1097/00024382-199506002-00224
130. Gonzalez, P. K.; Zhuang, J.; Doctrow, S. R.; Malfroy, B.; Smith, M.; Menconi, M. J.; Fink, M. P. Delayed treatment with EUK-8, a novel synthetic superoxide dismutase (SOD) and catalase (CAT) mimetic, ameliorates acute lung injury in endotoxemic pigs. *Surg. Forum,* **1995**, *46*, 72–73.
131. González, P. K.; Zhuang, J.; Doctrow, S. R.; Malfroy, B.; Benson P. F.; Menconi, M. J.; Fink, M. P. Role of oxidant stress in the adult respiratoy distress syndrome: evaluation of a novel antioxidant strategy in a porcine model of endotoxin-induced acute lung injury. *Shock* **1996**, *6*, S23-S26; DOI: 10.1097/00024382-199610001-00006
132. Kim, S. J.; Cheresh, P.; Jablonski, R. P.; Morales-Nebreda, L.; Cheng, Y.; Hogan, E.; Yeldandi, A.; Chi, M.; Piseaux, R.; Ridge, K.; Hart, C. M.; Chandel, N.; Budinger, G. R. S.; Kamp, D. W. Mitochondrial catalase overexpressed transgenic mice are protected against lung fibrosis in part via preventing alveolar epithelial cell mitochondrial DNA damage. *Free Radic. Biol. Med*. **2016,** *101*, 482-490; DOI: 10.1016/j.freeradbiomed.2016.11.007.

133. Mahmood, J.; Jelveh, S.; Calveley, V.; Zaidi, A.; Doctrow, S.R.; Hill, R.P. Mitigation of radiation-induced lung injury by genistein and EUK-207. *Int. J. Radiat. Biol.* **2011**, *87*, 889-901; DOI: 10.3109/09553002.2011.583315.
134. Mahmood, J.; Jelveh, S.; Zaidi, A.; Doctrow, S. R.; Medhora, M.; Hill, R. P. Targeting the renin–angiotensin system combined with an antioxidant is highly effective in mitigating radiation-induced lung damage. *Int. J. Radiat. Oncol. Biol. Phys.* **2014**, *89*, 722-728; DOI: 10.1016/j.ijrobp.2014.03.048.
135. Gao, F.; Fish, B. L.; Szabo, A.; Doctrow, S. R.; Kma, L.; Molthen, R. C.; Moulder, J. E.; Jacobs, E.; Medhora, M. Short-term treatment with a SOD/catalase mimetic, EUK-207, mitigates pneumonitis and fibrosis after single-dose total-body or whole-thoracic irradiation. *Radiat. res.* **2012,** *178*, 468-480; DOI: 10.1667/RR2953.1
136. Mathieu, E.; Bernard, A.-S.; Delsuc, N.; Quévrain, E.; Gazzah, G.; Lai, B.; Chain, F.; Langella, P.; Bachelet, M.; Masliah, J.; Seksik, P.; Policar, C. A cell-penetrant manganese superoxide dismutase (MnSOD) mimic is able to complement MnSOD and exerts an anti-inflammatory effect on cellular and animal models of inflammatory bowel diseases. *Inorg. Chem.* **2017**, *56*, 2545-2555; DOI: 10.1021/acs.inorgchem.6b02695.
137. Redl, H.; Gasser, H.; Schlag, G.; Marzi, I. Involvement of oxygen radicals in shock related cell injury. *Br. Med. Bull.* **1993**, *49*, 556-565; DOI: 10.1093/oxfordjournals.bmb.a072630.
138. van Empel, V. P.; Bertrand, A. T.; van Oort, R. J.; van der Nagel, R.; Engelen, M.; van Rijen, H. V.; Doevendans, P. A.; Crijns, H. J.; Ackerman, S. L.; Sluiter, W., De Windt, L. J. EUK-8, a superoxide dismutase and catalase mimetic, reduces cardiac oxidative stress and ameliorates pressure overload-induced heart failure in the harlequin mouse mutant. *J. Am. Coll. Cardiol*. **2006,** *48*, 824-832; DOI: 10.1016/j.jacc.2006.02.075
139. Srinivasan, V.; Doctrow, S.; Singh, V. K.; Whitnall, M. H. Evaluation of EUK-189, a synthetic superoxide dismutase/catalase mimetic as a radiation countermeasure. *Immunopharm. Immunot.* **2008,** *30*, 271-290; DOI: 10.1080/08923970801925331
140. García-Quintans, N.; Prieto, I.; Sánchez-Ramos, C.; Luque, A.; Arza, E.; Olmos, Y.; Monsalve, M. Regulation of endothelial dynamics by PGC-1α relies on ROS control of VEGF-A signaling. *Free Radic. Biol. Med.* **2016**, *93*, 41-51; DOI: 10.1016/j.freeradbiomed.2016.01.021
141. Himori, K.; Abe, M.; Tatebayashi, D.; Lee, J.; Westerblad, H.; Lanner, J. T.; Yamada, T. Superoxide dismutase/catalase mimetic EUK-134 prevents diaphragm muscle weakness in monocrotalin-induced pulmonary hypertension. *PloS one.* **2017**, *12*, e0169146; DOI: 10.1371/journal.pone.0169146
142. Atkinson, T.; Packwood, W.; Xie, A.; Liang, S.; Qi, Y.; Ruggeri, Z.; Lopez, J.; Davidson, B. P.; Lindner, J. R. Assessment of novel antioxidant therapy in atherosclerosis by contrast ultrasound molecular imaging. *J. Am. Soc. Echocardiog.* **2018**, *31*, 1252-1259; DOI: 10.1016/j.echo.2018.07.017
143. Nishigori, C.; Hattori, Y.; Arima, Y.; Miyachi, Y. Photoaging and oxidative stress. *Exp. Dermatol.* **2003**, *12*, 18-21; DOI: 10.1034/j.1600-0625.12.s2.3.x
144. Tocco, G.; Illigens, B. M. W.; Malfroy, B.; Benichou, G. Prolongation of alloskin graft survival by catalytic scavengers of reactive oxygen species. *Cell. Immunol.* **2006**, *241*, 59-65; DOI: 10.1016/j.cellimm.2006.06.009
145. Kvale, E.O.; Patel, N. S. A.; Chatterjee, P. K.; Sharpe, M. A.; Thiemermann, C. The SOD mimetic EUK-134 reduces oxidative stress-mediated renal dysfunction in the rat in vivo. *Br. J. Pharmacol*. **2002**, *135*, 69P.
146. Decraene, D.; Smaers, K.; Gan, D.; Mammone, T.; Matsui, M.; Maes, D.; Declercq, L.; Garmyn, M. A synthetic superoxide dismutase/catalase mimetic (EUK-134) inhibits membrane damage-induced activation of mitogen-activated protein kinase pathways and reduces p53 accumulation in ultraviolet B-exposes primary human keratinocytes. *J. Invest. Dermatol.* **2004**, *122*, 484-491; DOI: 10.1046/j.0022-202X.2004.22215.x
147. Doctrow, S. R.; Lopez, A.; Schock, A. M.; Duncan, N. E.; Jourdan, M. M.; Olasz, E. B.; Moulder, J. E.; Fish, B. L.; Mäder, M.; Lazar, J.; Lazarova, Z. A synthetic superoxide dismutase/catalase mimetic EUK-207 mitigates radiation dermatitis and promotes wound healing in irradiated rat skin. *J. Investig. Dermatol.* **2013,** *133*, 1088-1096; DOI: 10.1038/jid.2012.410
148. Jelveh S.; Kaspler P.: Bhogal N.; Mahmood J.; Lindsay P.E; Okunieff P.; Doctrow S.R.; Bristow R.G.; Hill R.P. Investigations of antioxidant-mediated protection and mitigation of radiation-induced DNA damage and lipid peroxidation in murine skin. *Int. J. Radiat. Biol.* **2013**, *89*, 618–27; DOI: 10.3109/09553002.2013.782450
149. Laforgia, N.; Di Mauro, A.; Guarnieri, G. F.; Varvara, D.; De Cosmo, L.; Panza, R.; Capozza; Baldassarre, M. E.; Resta, N. The role of oxidative stress in the pathomechanism of congenital malformations. *Oxid. Med. Cell Longev.* **2018**, 7404082; DOI: 10.1155/2018/7404082

150. Hu, X. Q.; Huang, X.; Xiao, D.; Zhang, L. Direct effect of chronic hypoxia in suppressing large conductance Ca^{2+}-activated K^+ channel activity in ovine uterine arteries via increasing oxidative stress. *J. physiol*. **2016**, *594*, 343-356; DOI: 10.1113/JP271626
151. Chen, S. Y.,; Dehart, D. B.; Sulik, K. K. Protection from ethanol-induced limb malformations by the superoxide dismutase/catalase mimetic, EUK-134. *The FASEB journal*, **2004,** *18*, 1234-1236; DOI: 10.1096/fj.03-0850fje
152. Zhang, H. J.; Doctrow, S. R.; Xu, L.; Oberley, L. W.; Beecher, B.; Morrison, J.; Kregel, K. C. . Redox modulation of the liver with chronic antioxidant enzyme mimetic treatment prevents age-related oxidative damage associated with environmental stress. *The FASEB journal*, **2004,** *18*, 1547-1549; DOI: 10.1096/fj.04-1629fje
153. Rezazadeh, A.; Yazdanparast, R.; Molaei, M. Amelioration of diet-induced nonalcoholic steatohepatitis in rats by Mn-salen complexes via reduction of oxidative stress. *J. biomed. Sci.* **2012,** *19*, 26; DOI: 10.1186/1423-0127-19-26
154. Meftah, S.; Yazdanparast, R.; Molaei, M. Ameliorative Action of Mn-Salen Derivatives on CCl_4-Induced Destructive Effects and Lipofuscin-Like Pigment Formation in Rats' Liver and Brain: Post-Treatment of Young Rats with EUKs. *CellBio*. **2014**, *3*, 96; DOI: 10.4236/cellbio.2014.33010
155. Magder, S.; Parthenis, D. G.; Ghouleh, I. A. Preservation of Renal Blood Flow by the Antioxidant EUK-134 in LPS-Treated Pigs. *Int. J. Mol. Sci*. **2015,** *16*, 6801-6817; DOI: 10.3390/ijms16046801
156. Harman, D.; Aging: a theory based on free radical and radiation chemistry. *J Gerontol*. **1956**, *11*, 298–300; DOI: 10.1093/geronj/11.3.298
157. Kirkwood, T. B. L.; Kowald, A. The free-radical theory of ageing – older, wiser and still alive: modelling positional effects of the primary targets of ROS reveals new support. *Bioessays* **2012**, *34*, 692-700; DOI: 10.1002/bies.201200014
158. Stadtman, E. R. Protein oxidation and aging. *Science* **1992**, *257*, 1220-1224; DOI: 10.1126/science.1355616
159. Gladyshev, V. N. The free radical theory of aging is dead. Long live the damage theory! *Antioxid. Redox Signal.* **2014**, *20*, 727-731; DOI: 10.1089/ars.2013.5228
160. Van Raamsdonk, J. M.; Hekimi, S. Deletion of the mitochondrial superoxide dismutase sod-2 extends lifespan in Caenorhabditis elegans. *PLoS Genet.* **2009**, *5*, e1000361; DOI: 10.1371/journal.pgen.1000361
161. Keaney, M.; Matthijssens, F.; Sharpe, M.; Vanfleteren, J.; Gems, D. Superoxide dismutase mimetics elevate superoxide dismutase activity in vivo but do not retard aging in the nematode *Caenorhabditis elegans*. *Free Radic. Biol. Med.* **2004**, *37*, 239-250; DOI: 10.1016/j.freeradbiomed.2004.04.005
162. Sampayo, J. N.; Olsen, A.; Lithgow, G. J. Oxidative stress in *Caenorhabditis elegans*: protective effects of superoxide dismutase/catalase mimetics. *Aging cell.* **2003**, *2*, 319-326; DOI: 10.1046/j.1474-9728.2003.00063.x
163. Magwere, T.; West, M.; Riyahi, K.; Murphy, M. P.; Smith, R. A.; Partridge, L. The effects of exogenous antioxidants on lifespan and oxidative stress resistance in *Drosophila melanogaster*. *Mech. ageing dev.* **2005**, *127*, 356-370; DOI: 10.1016/j.mad.2005.12.009
164. Bayne A.-C. V.; Sohal R. S. Effects of superoxide dismutase/catalase mimetics on life span and oxidative stress resistance in the housefly, *Musca Domestica*, *Free Radic. Biol. Med.* **2002**, *32*, 1229–1234; DOI: 10.1016/S0891-5849(02)00849-3
165. Watanabe, D.; Aibara, C.; Wada, M. Treatment with EUK-134 improves sarcoplasmic reticulum Ca2+ release but not myofibrillar Ca2+ sensitivity after fatiguing contraction of rat fast-twitch muscle. *Am. J. Physiol.-Reg. I.* **2019**, *316*, R543-R551; DOI: 10.1152/ajpregu.00387.2018
166. Arndt, A.; Borella, M. I.; Espósito, B. P. Toxicity of manganese metallodrugs toward Danio rerio. *Chemosphere*. **2014**, *96*, 46-50; DOI: 10.1016/j.chemosphere.2013.07.014
167. Grodstein, F.; O'Brien, J.; Kang, J. H.; Dushkes, R.; Cook, N. R.; Okereke, O.; Sesso, H. D. Long-term multivitamin supplementation and cognitive function in men: A randomized trial. *Ann. Intern. Med.* **2013**, *159*, 806-814; DOI: 10.7326/0003-4819-159-12-201312170-00006
168. Oertel, W.; Schulz, J.B. Current and experimental treatments of Parkinson disease: A guide for neuroscientists. *J. Neurochem.* **2016**, *139*, 325-337; DOI: 10.1111/jnc.13750
169. Shen, L.; Hong-Fang, J. Is antioxidant supplement beneficial? New avenue to explore. *Trends in Food Sci. Tech.* **2017**, *68*, 51-55; DOI: 10.1016/j.tifs.2017.08.010