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Superoxide dismutases: a physiopharmacological update

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Reactive oxygen species (ROS) are known participants in several cellular processes. Superoxide anion radical, one example of ROS, forms as a result of normal cellular respiration and is usually cleared successfully by superoxide dismutase (SOD) and other radical scavengers. However, when superoxide exceeds the clearance capacity of SOD and other ROS scavengers, superoxide initiates a number of pathologic processes. This review examines pathologies involving superoxide, including: cancer, neurodegenerative diseases, ischemia/reperfusion injury, and inflammation. We will also explore the basic science principles of superoxide and SOD, including: SOD evolution, SOD mutations, biochemistry, physiology, and pathophysiology. In reviewing the basic science, clinical pathology, and therapeutic research, we hope to clearly demonstrate plausible pharmacologic targets of action. We have revised data about basic science, clinical pathology and therapeutic research in an effort to propose plausible pharmacological targets of action. The understanding of these aspects is critical in the accomplishment of a successful clinical intervention.

Key words: Superoxide, Oxidative stress, Mitochondria, Apoptosis.

Although oxygen is central to life, metabolic utilization of this gas also results in the production of the undesirable by-product superoxide, a reactive oxygen species (ROS). In fact, aerobic organisms survive the presence of harmful ROS only because they contain antioxidant defenses. Antioxidants are molecules or compounds that act as free radical scavengers. These cellular anti-oxidant systems regulate the levels of ROS. During healthy aerobic respiration, the production of reactive species is approximately balanced with antioxidant defense sys-

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tems. The balance between ROS production and antioxidant capacity is delicate, and its disruption results in oxidative stress (79). By definition, oxidative stress exists when free radical formation is in excess relative to protective antioxidants.

The group of ROS includes superoxide ($\bullet O_2^-$), hydroxyl radicals (•OH), singlet O, and hydrogen peroxide (H_2O_2) (41, 100). ROS may be generated intrinsically via cellular metabolism, phagocytosis, mitochondrial respiration, and xenobiotic detoxification. Exogenous sources include ionizing radiation and chemical compounds performing red-ox reactions.

The superoxide radical anion ($\bullet O_2^-$) is an initial form of metabolically produced ROS (72), formed when oxygen acquires one electron. An initial production site is the internal mitochondrial membrane, involving enzymes such as NADH ubiquinone reductase and ubiquinone cytochrome C reductase. There is also production of $\bullet O_2^-$ at the cellular membrane level. For example, phagocytic macrophages utilizing NADPH oxidase to produce a bactericidal oxidative burst. However, $\bullet O_2^-$ can be so toxic that intracellular levels above 1nM are lethal. The toxicity of $\bullet O_2^-$ might derive from its capacity to inactivate iron-sulfur containing enzymes, and initiate lipid peroxidation of polyunsaturated fatty acids. Further damage is perpetuated when $\bullet O_2^-$ reacts with carbonyl compounds and halogenated carbons to create more highly reactive radicals, as when superoxide combines with nitric oxide (NO) to form peroxynitrite $ONOO^-$ (22). As such, $\bullet O_2^-$ is one of the main instigators of cellular oxidative stress. Furthermore, in an early theory about aging, Harman proposed a central role for superoxide radicals (42). The list of pathophysiological conditions that are associated with the overproduction of $\bullet O_2^-$ expands every day. Superoxide participates in many neurodegenerative processes including: withdrawal of nerve growth factor to sympathetic neuronal cultures (53), veratridine-induced cell death (52), and ischemic activation of p53 (51). In addition, $\bullet O_2^-$ is able to activate the formation of the mitochondrial permeability pore (29), which has been considered as the point-of-no-return in apoptotic pathways (49).

Interestingly, $\bullet O_2^-$ does not readily cross cellular membranes and, consequently, there are distinct extracellular, cytosolic, and mitochondrial pools of superoxide (75, 77). These various sources of superoxide can affect living organism in a variety of ways. While $\bullet O_2^-$ itself is not sufficiently reactive to directly damage DNA (40), it can facilitate DNA damage by interacting with free iron and hydrogen peroxide to form more reactive hydroxyl radicals. In the iron-sulfur containing enzyme aconitase, $\bullet O_2^-$ promotes the release of iron from iron-sulfur clusters (68, 55). Important tissue damaging and pro-inflammatory roles attributed to $\bullet O_2^-$ include: endothelial cell damage and increased microvascular permeability (20, 39, 111), formation of chemotactic factors such as leukotriene B_4 (18, 23), recruitment of neutrophils at sites of inflammation (10, 94, 95), lipid peroxidation and oxidation, and single-strand DNA damage (19). Superoxide anion radicals contribute to post-ischemic reperfusion tissue damage demonstrated in several organs (3, 34, 37, 76, 115).

The superoxide dismutase family (SOD) catalyzes the conversion of $\bullet O_2^-$ to H_2O_2 and O_2, reducing the dangerous presence of $\bullet O_2^-$ (5). The SOD-mediated mechanism involves the reduction and cyclical re-oxidation of Cu^{2+} by the molecules of $\bullet O_2^-$ (107).

SOD family members

The discovery of the enzymatic activity of the SODs was reported in 1968 by McCord and Fridovich (73). However, the proteins had already been twice described 30 years earlier in bovine blood and liver when Mann and Keilin (71) purified a copper-binding protein of unknown function. This protein was called "erythrocuprein" or "hepatocuprein," and later "cytocuprein" Four classes of SODs are known, distinguished by the metal prosthetic group: Cu/Zn, Mn/Ni, Fe- and Mn-SODs (82).

The intracellular Cu/Zn SOD, or SOD1 isoform, is present in the cytoplasm, nuclear compartment, and plasma. The human SOD-1 gene has been localized to chromosome 21q22 in humans, composed of five exons and four introns, and its promoter contains several putative binding sites for NF1, Sp1, AP1, AP2, GRE, HSF, and NF-κβ transcription factors. This SOD family member is a functional dimer with a weight of 32 kDa, containing an ion of copper and zinc as a subunit. The structure of the active binding site in the oxidative form of the protein was investigated by crystallographic studies in mammals, amphibians, fungi, and bacteria. In all cases, the copper and zinc ions are linked by the imidazole ring of histidine 61 residue, which coordinates both metals. Copper is coordinated by four histidine residues and a water molecule, utilizing a square pyramidal symmetry for binding. Zinc is coordinated by three histidine and one aspartate residue, binding in a tetrahedral geometry (45, 46).

The Fe-SODs is unequally distributed throughout the kingdoms of living organisms and is located in different cellular compartments (36). Fe-SOD is found in obligate anaerobes and aerobic diazotrophs, facultative aerobes, the cytosol of cyanobacteria, the chloroplast stroma of higher plants, and in protozoa. The 3–D structures of several Fe-SODs have been determined (65, 96). The monomers fold into two domains. The N–terminal domain consists of two antiparallel α-helices, while the C-terminal domain contains a central βsheet formed by three antiparallel βstrands with 4-6 surrounding α-helices. The iron atom is coordinated by two residues from each of the N-terminal α-helices and two residues from the loops of the C–terminal domain. In the active site iron is pentacoordinate, with the metal ligand (N of three conserved His residues, O of the conserved Asp residue, and a water molecule) arranged in a distorted trigonal bipyramidal geometry. The first His residue and a solvent molecule fill the two axial positions. In the azide-FeIII-SOD complex, iron becomes hexacoordinate with a distorted octahedral geometry with the azide portion of an aspartate residue located *trans* to the ligand (63).

The Mn-SOD isoform (SOD2) is primarily located in mitochondria and is considered the primary isoform in relation to oxidative stress. The human SOD2 gene, with five exons and four introns, was localized to chromosome 6q25. The SOD2 promoter region lacks TATA and CAAT boxes, and contains putative Sp1, AP2 and NF-κβ transcription regulatory elements. This indicates that SOD2 is induced by multiple stress conditions, both *in vitro* and *in vivo*, by exposure to high oxygen tension (12, 44), ozone (110), cigarette smoke (33), chronic hypoxia (101), cytokines (TNF-α, IFN-γ, IL-1, IL-6), lipopolysaccharide, asbestos fibers, radiation (2, 38), and modulators of intracellular redox and/or thiol state (15, 16, 61, 67, 99, 109). The encoded protein is a homotetramer of 96 kDa that contains a

Mn atom at each subunit. Although the contents of Mn-SOD in human tissue is about half the contents of Cu/Zn-SOD, the expression of Mn-SOD is essential for the survival of aerobic life and the development of cellular resistance to the toxicity created by ROS. 2-4% o f the oxygen consumed by mitochondria during the transport of electrons forms the semiquinone anion which can transform molecular oxygen to $\bullet O_2^-$ instead of making water by using cytochrome C oxidase.

The extracellular SOD (EC-SOD or SOD3) contains Cu and Zn, and is extracellularly released. The SOD3 gene promoter region lacks TATA and CCAAT boxes, but includes a metal regulatory element, an AP1 site, and two potential antioxidant response elements (27). Its genomic structure and chromosomal localization gene have been mapped in humans to chromosome 4 4q21. This SOD family member is a hydrophobic glycoprotein with molecular weight of approximately 135 kDa. In most species, EC-SOD is a tetramer of identical 30 kDa subunits, linked through disulfide bridges, although it is occasionally found as a dimmer (24, 79-81). In contrast to SOD1 and SOD2, the expression of EC-SOD appears restricted to only a few cell types in several tissues (e. g. alveolar type II (25) and vascular smooth muscle cells (104).

Evolution of SOD genes

As previously mentioned, SOD enzymes are among the most important defenses against oxygen radicals. In fact, the origin of SOD genes is supposed to be about 2 billion years ago, coinciding with the proliferation of photosynthetic organisms that began to produce oxygen (117). The human SOD family is derived from genes for the iron/manganese- containing

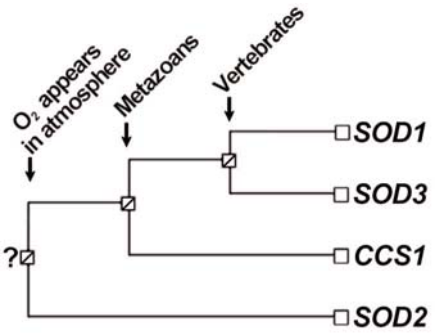

Fig. 1. *Evolutionary tree of SOD gene family.* Dashed squares represent gene duplication nodes that diverged into two genes. Question mark indicates an unknown common ancestral gene. Arrows indicate evolutionary changes when each gen divergence ocurred.

SOD2, copper/zinc-containing SOD1, SOD3, and CCS1 (a copper chaperone) (Fig. 1). It is unknown if SOD2 shares a common ancestor with the other three genes. It is possible that all SODs originated from an ancestral oxygen protective enzyme, although structural characteristics and functional enzymatic mechanisms suggest that SOD2 could have a different origin. In the Cu/Zn SODs evolutionary branch, the copper chaperone CCS1 (involved in copper transport to Cu/Zn SODs) diverged at the origin of metazoans (the animal kingdom) and is highly conserved (64). SOD3 is more similar to SOD1 of the same phylum than to the SOD3 orthologs of other phyla (4). While similarities could be due to an abnormal evolutionary rate of these genes (117), this finding strongly suggests that the divergence between these genes occurred independently and multiple times by the addition of a signal peptide to cytoplasmic SOD1 (64).

Phylogenic genetic trees of SOD obtained from the Ensembl Project (Fig. 2) match the currently known phylogenetic relationships amongst species. Discordances at high taxonomic levels

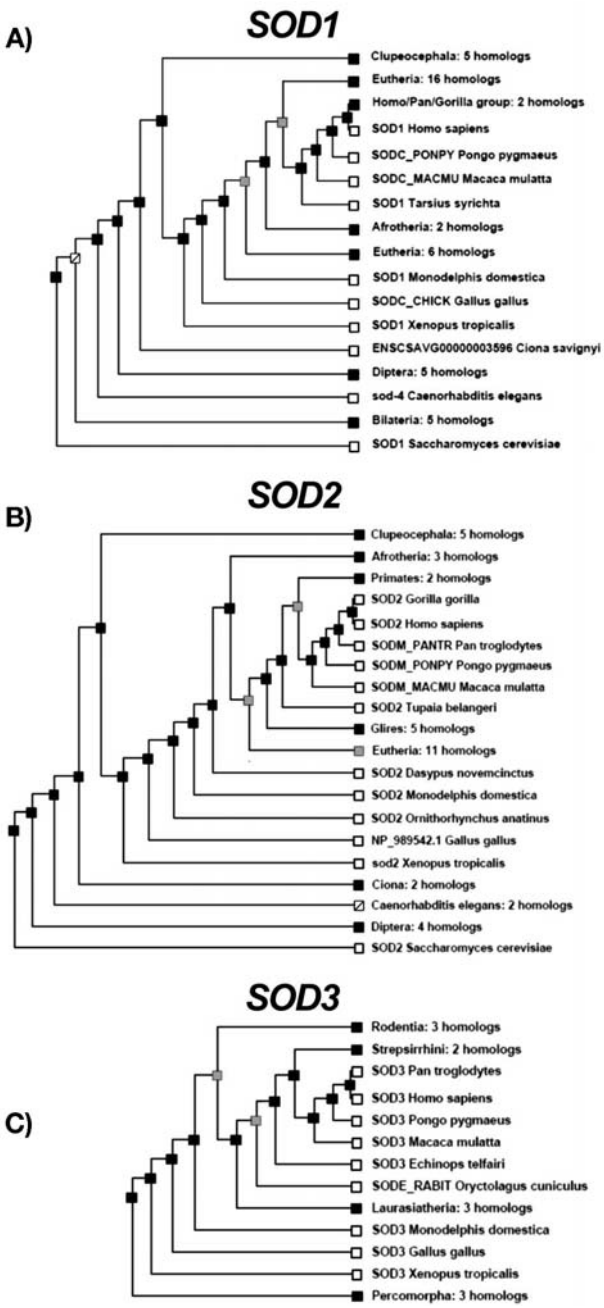

Fig. 2. *Phylogenic trees of SOD genes.* Phylogenic relationships between SOD1 (A), SOD2 (B) and SOD3 (C) orhtologs are based on amino acid sequence comparisons obtained from the Ensemble Project (http://www.ensembl. org). Dashed squares = gene duplication nodes, black squares = speciation nodes, and gray squares = ambiguous nodes.

(ambiguous nodes) could be due to rapid genomic divergence of N- and C- terminal ends of Cu/Zn SOD (85), or incomplete sequences in the database. Most of the higher eukaryotes have only one ortholog for SOD1. However, there have been some pseudogenes in several species as mice, *Drosophila melanogaster*, and *Anopheles gambiae* (64).

Physiological role

Under normal circumstances, intrinsic SOD enzymes catalytically remove superoxide by converting it into oxygen and hydrogen peroxide. Hydrogen peroxide is further processed by catalase to form water and oxygen. Nitric oxide (NO) is normally a vasodilatory agent, but can combine with superoxide to generate toxic metabolites such as peroxynitrite. Thus, SODs are intimately involved in the regulation of $\bullet O_2^-$, H_2O_2, and the metabolites of nitrogen (59). It was shown that SOD is rapidly incorporated into several cell types in the lung after intratracheal administration, preventing inflammation and acute lung injury induced by mechanical ventilation and hyperoxia (17). As the main antioxidant in the eye, clinical studies have suggested that SODs could play an important role in preventing the formation of cataracts (66). In addition to its role in disease states, EC-SOD may also play a role in the transition from fetal to adult circulation, as the secretion of active EC-SOD is regulated in the developing lung after birth (78). Finally EC-SOD also attenuates inflammation and neutrophil influx in lipopolysaccharide (LPS) exposure models (25). One possible mechanism is that the EC-SOD/collagen interaction in the extracellular matrix could lessen the production of collagen fragments. With fewer collagen fragments acting as chemoattractants for neutrophils, the inflammatory reaction would be diminished (79).

SOD in pathology

Oxidative stress that results from the production and reactivity of ROS has emerged as a main pathogenic event in human diseases. In disease states such as inflammation, the immune system produces an excess of superoxide overwhelming the ability of native SOD to remove free radicals. An excess of free radicals ultimately results in damage to cells and tissues. Superoxide damage has been associated with a growing number of diseases and conditions such as cancer, neurodegenerative diseases, ischemia/reperfusion injury, and inflammation.

1. Cancer

There is a great controversy with regard to the function of SOD in cancer. Many human tumors have been shown to express high levels of SODs, and this has been associated with aggressive tumor characteristics. However, other studies have found low SOD activity in the same or different classes of tumors (58). Low expression of Mn-SOD has been observed in different cancer tissues, and several reports have shown that overexpression of Mn-SOD inhibits growth in various human cancer cells. These observations suggest that a reduced concentration or fuction of Mn-SOD is involved in carcinogenesis. It has been shown that a DNA point mutation of T by C in the mitochondrial targeting sequence (MTS) of Mn-SOD results in the substituion of valine (Val) with alanine (Ala) at position 16 (87, 98). This amino acid replacement has been suggested to change the mitochondrial targeting of the enzyme, there-

by influencing cellular defenses against superoxide radicals. Furthermore, the -9Ala/-9Val polymorphism in the MTS of Mn-SOD is associated with a significantly earlier onset of colorectal carcinoma among Hispanics (103).

Recently it has been shown that the Ala MTS polymorphism of Mn-SOD allows efficient targeting of Mn-SOD to the mitochondria, whereas the Val variant leads to a decreased formation of active Mn-SOD in the mitochondrial matrix (106). In a case-control study, BERGMAN *et al.* (9) suggested that Mn-SOD may be implicated in breast carcinogenesis in young women since individuals with Mn-SOD (Val/Val) and Mn-SOD (Val/Ala) genotypes showed an increased risk of breast cancer. Moreover, 45% of the informative cases expressed allelic loss at the chromosomal locus of the Mn-SOD gene (9).

Another polymorphism of Mn-SOD (Thr58Ile) has been reported (43). This polymorphism may also have effects on the expression of Mn-SOD activity. In cultured breast carcinoma cells, transfection of the Ile form results in a 3-fold increase in Mn-SOD activity when compared to the Thr polymorphism (118). In addition, three intronic polymorphisms and three polymorphisms in the promoter region of the Mn-SOD gene have been described. *In vitro* changes in the promoter region of Mn-SOD can alter the pattern of AP-2 binding and transcriptional activity (54), as seen in prostate cancer (n = 1,320) with genetic polymorphisms in Cu/Zn-SOD (IVS3-251A>G), Mn-SOD [Ex2+24T>C (V16A)], and EC-SOD; (IVS1+186C>T, Ex3-631C>G, Ex3-516C>T, and Ex3-489C>T). The more active Ala variant of Mn-SOD (Ex2+24T>C (V16A)), which has been hypothesized to suppress prostate carcinogenesis, was associated with an elevation of prostate cancer risk in Caucasians. However, no significant association with prostate cancer was observed for polymorphic variants in EC-SOD or Cu/Zn-SOD (54).

2. Neurodegenerative diseases

The free radical theory was thought to be one of the mechanisms involved in the pathogenesis of several neurodegenerative diseases including: Parkinson's disease (PD), Alzheimer's disease (AD), and amyotrophic lateral sclerosis (ALS). An excess of free radicals beyond the capabilities of intrinsic SOD upsets a delicate cellular balance that ultimately contributes to neurodegeneration.

Parkinson's disease.– Oxidative stress is produced when there is an increased formation or defective clearance of cytotoxic reactive oxygen species (ROS). Excess ROS can initiate and/or promote the degeneration of dopaminergic neurons in PD patients. There are reports suggesting a decrease in SOD and other antioxidant enzyme activity, with increases in various markers of lipid peroxidation in the substantia nigra in PD (1, 35, 56). Patients with PD had significantly higher SOD activity within the red blood cell (RBC). The mean RBC activity of catalase in PD did not differ significantly from those of controls, but was significantly lower in the more advanced cases of PD when compared to early cases. Oxidized glutathione was significantly higher in RBCs of PD patients, although there were no changes in total glutathione and reduced glutathione compared to controls. Thiobarbituric acid reactive substances (TBARS) content was increased in patients with PD (116). The increase in the oxidative stress due to the low activity of

antioxidant enzymes might pave the way for many secondary complications, potentially contributing to the neurodegeneration seen in PD (1). On the other hand, it has been reported that Cu/Zn-SOD could be a source of copper and oxidative stress that might trigger the pathological aggregation of α-synuclein (57). Therefore, the function of Cu/Zn-SOD as a free radical generator might be related to detrimental oxidation reactions, which may play a critical role in neurodegenerative disorders. Recently, it was reported that the aggregation of α-synuclein was induced by the Cu/Zn-SOD/H_2O_2 system (57). While SOD helps clear the dangerous superoxide ROS, the hydrogen peroxide byproducts as another ROS still pose a threat to cells in the absence of adequate catalase activity.

Alzheimer's disease.– The involvement of oxidative stress as a causal event in the age-associated development of AD pathology has been suggested (32, 70, 105). Studies carried out with amyloid precursor protein (APP) transgenic mice, specifically Thy1-APP751SL transgenic mice with a higher β-amyloid (Aβ) load, are affected by increased lipid peroxidation possibly related to impaired Cu/Zn-SOD activity (97). Recent studies suggest that Mn-SOD plays a protective role during AD development. For example, Mn-SOD deficiency increases Aβ levels and the amyloid plaque burden, and accelerates the onset of behavioral alterations in APP transgenic mice (21, 69). Mutations in the APP and presenilin (PS) genes increase oxidative stress in APP/PS1 neurons, and result in increased Mn-SOD levels which act as an adaptive antioxidant response. However, sustained exposure to high levels of oxidative stress is accompanied by a decline in Mn-SOD production and increased vulnerability to β-amyloid exposure (102).

Amyotrophic lateral sclerosis.– ALS is a lethal disease that results from the progressive death of motor neurons in the corticospinal tracts and brain stem, resulting in rapid muscle degeneration and paralysis. Although the precise etiology of ALS remains unclear, mutations in the SOD1 gene are known to account for approximately 20% of familial ALS (FALS). In 1993, 13 mutations to the cytosolic Cu/Zn-SOD were discovered in about 2–3% of individuals with ALS, with ~90 different SOD mutations now reported. The vast majority of these mutations are missense point mutations, although a few deletions and insertions have been reported in the C-terminal region (8). The SOD mutations in individuals with ALS are dominant, which suggests they confer a toxic gain of function, rather than simply diminishing superoxide-scavenging activity. The Ala4Val mutant of Cu/Zn-SOD (A4V) is the most common Cu/Zn-SOD mutation discovered to date, accounting for ~50% of Cu/Zn-SOD-linked FALS cases. The A4V point mutation is a particularly severe mutation in that it induces a rapid rate of disease progression, resulting in death within an average of 1. 2 years after the onset of symptoms (86). Crystal structure analysis of the Ala4Val (A4V) mutant to 1. 9 Å revealed small changes at the dimer interface, resulting in a substantial reorientation of the two monomers. We have previously shown that the expression of two FALS-related mutant SODs (A4V and V148G) caused the death of differentiated PC12 cells, superior cervical ganglion neurons, and pyramidal neurons

within the hippocampus. Cell death included many features typical of apoptosis. Death could be prevented by copper chelators, Bcl-2, glutathione, vitamin E, and caspase inhibitors (31).

3. Ischemia/reperfusion

Numerous studies have documented the ability of exogenous SOD1 to reduce tissue injury following temporary ischemia/reperfusion. Neutrophil activation at sites of injury results in a large production of $\bullet O_2^-$, which in turn contributes to tissue damage seen post-reperfusion in several ischemic organs including: kidneys (76), stomach (114), skin (34), and heart (3). Cu/Zn-SOD prevents vasogenic brain edema after several kinds of injuries (60), suggesting that $\bullet O_2^-$ is an important factor for blood–brain barrier disruption (62). Acute hypoxia diminishes Mn-SOD mRNA expression in part by decreasing mRNA stability *in vitro* (48), and possibly Mn-SOD protein synthesis *in vivo* (88). Thus Mn-SOD may be down-regulated during severe oxidant/nitrative stress and acute hypoxia. Exogenous SOD1 treatment is beneficial in several experimental models of head trauma and central nervous system (CNS) ischemia/reperfusion (28). Analogously, transgenic mice overexpressing wild-type human SOD1 demonstrate reduced brain damage following temporary ischemia/reperfusion (60). However, a potential limitation in the efficacy of SOD1 in this setting is its inability to permeate cells; neurons and astrocytes do not appear to take up the native enzyme under normal conditions (11, 89).

4. Inflammation

Some important tissue damaging and pro-inflammatory roles attributed to $\bullet O_2^-$ include: endothelial cell damage with increased microvascular permeability (20, 39, 111), formation of chemotactic factors such as leukotrienes B_4 (18, 23), recruitment of neutrophils at sites of inflammation (10, 92, 94, 95), lipid peroxidation and oxidation, and DNA single-strand damage (19). The product formed as a result of $\bullet O_2^-$ interacting with NO is peroxynitrite ($ONOO^-$), a potent, cytotoxic, pro-inflammatory molecule (6, 7, 13, 47, 74, 92, 93, 95). By interacting with NO, $\bullet O_2^-$ destroys the biological activity of this mediator, attenuating important anti-inflammatory and tissue protective properties. Superoxide attenuates the effects of NO on: maintenance of blood vessel tone and platelet reactivity, cytoprotective effect in numerous organs (including heart, intestine, and kidneys), and release of anti-inflammatory and cytoprotective prostacyclin (via activation of the constitutive cyclo-oxygenase enzyme) (90, 91). Therefore, removal of superoxide allows NO to address environments of cellular stress and reduces the formation of cytotoxic $ONOO^-$. Finally, EC-SOD also attenuates inflammation and neutrophil influx in lipopolysaccharide (LPS) exposure models (26).

Therapeutic approximation

Evidence from cellular and animal models supports the hypothesis that SOD plays an integral role in the degenerative process, and implies that SOD-based therapies should be considered for the treatment of several illnesses. Because of their importance, the SODs have received much attention in efforts to minimize oxygen radical-induced tissue damage. Since the administration of exogenous SODs has often proven to be problematic,

Table I. *Beneficial effects of SOD mimetics on oxidative stress models.*

SOD mimetic	Model	Reference
EUK-8	Myocardial oxidant damage	108
EUK-134	Paraquat-mediated SNpc dopaminergic neuronal cell death	84
	Hyperoxygenation status after ischemia/reperfusion	112
	Suppression of mitochondrial respiration induced by peroxynitrite	119
EUK-189	Paraquat-mediated SNpc dopaminergic neuronal cell death in PD	83
MnTBAP	Inflammation-induced PARS activation	
	Carrageenan-induced paw edema	
	Veratridine-induced cell death	14
	6-Hydroxydopamine	30
M-40403	Rat models of inflammation and ischemia/reperfusion injury	96
	Co therapy with IL-2 for the treatment of skin and kidney cancers	
	Inflammation	
	Ischemia/reperfusion injury	
SC-55858	LPS -induced increase in microvascular leakage, lipid peroxidation and epithelial cell injury	93
Recombinant adenovirus human Cu/Zn-SOD (Ad-SOD)	Ischemia/reperfusion injury in the rat kidney	113
	Staurosporine-induced neuronal apoptosis	85
	Death of sympathetic neurons caused by withdrawal of nerve growth factor	53
	X-irradiation-induced neuronal death	50
M40403	Rat models of inflammation and ischemia-reperfusion injury	96

a variety of innovative approaches are currently being explored in conjunction with radiotherapy. Selective SOD mimetics are potentially useful in the above pathological conditions. A major step forward in this field was the development of small-molecule selective SOD mimetics that penetrate cell membranes. These selective SOD mimetics catalytically remove $\bullet O_2^-$ without interfering with nitric oxide (NO), peroxynitrite ($ONOO^-$), or other radicals such as hydroxyl radicals or H_2O_2. Among these SOD mimetics are: EUK-8, EUK-134 and EUK-189, EUK-207 refer to as "synthetic catalytic scavengers the porfirinins MnTBAP and SC-52608, SC-55858, M-40403 and M-40401). We and others have demonstrated the benefits that these selective SOD mimetics have shown in several animal models, some of them summarized in Table I.

SOD mimetics are well suited for use as drugs from a pharmacologic standpoint. SOD mimetics have a much lower molecular weight than the native enzyme, are able to permeate cells and membranes easily, are much more stable, have a longer half-life than native SODs, and do not elicit an immune response in the body. Furthermore, the SOD mimetic family successfully reproduces the beneficial and highly selective action of the natural enzyme.

These new, low-molecular-weight, synthetic Mn-SODs represent a breakthrough in chemical design. They are stable *in vivo*, possess high activity, and are selective for superoxide without activity towards H_2O_2, $ONOO^-$, NO, or OCl^-. Its selectivity resides in the nature of the

manganese (II) center in these low-molecular-weight complexes. The resting oxidation state of the complex is the reduced state, Mn(II). As a consequence, the complex has no reactivity with reducing agents until it is oxidized to Mn(III) by superoxide. Since the complex is so difficult to oxidize many oxidants will not oxidize these complexes, including nitric oxide and oxygen. Since they operate via a facile one-electron oxidation pathway, other two-electron non-radical oxidants (e. g. , $OONO^-$, H_2O_2, OCl^-) are also not able to oxidize the Mn(II) complex. The unique selectivity of these mimetics for superoxide despite the presence of other ROS makes it possible to discern the role of superoxide in disease models in which ROS are implicated. We have continued our computer-aided design and synthesis program, and have recently developed M40401 (the S,S-dimethyl substituted derivative of the M40403 biscyclohexylpyridyl class of mimetic). M40401 possesses a much higher catalytic activity at pH=7. 4. In fact, its catalytic rate exceeds $1x10^{+9}M^{-1}s^{-1}$, which is about 100 times the activity of M40403 at pH=7. 4, and on par with native Cu/Zn-SOD enzymes. As with M40403, M40401 has no catalase activity or reactivity with peroxynitrite.

Conclusions

These circumstantial data from the laboratory delineate the integral role of SOD in mediating neurodegenerative processes. There exists a need for quantitative clinical studies to determine whether SOD represents an effective entity to deal with the oxidative stress in human disease. Reanalysis of the data from previous trials could provide information to create better experimental designs, but experimental designs developed to observe direct SOD effects may be inadequate.

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Palabras clave: Anion superóxido, Estrés oxidativo, Mitocondria, Apoptosis.

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